THE
AMERICAN NATURALIST

VOL. LXIV *May–June, 1930* NO. 692

THE INTENSITY FACTOR IN VISION AND RADIATION[1]

PROFESSOR SELIG HECHT
THE LABORATORY OF BIOPHYSICS, COLUMBIA UNIVERSITY

I

LIGHT—or what is now fashionably called radiation—varies in two ways: in frequency and in intensity. Our eyes, and those of most other animals, are sensitive to only a very small range of frequencies, namely, to those between 400 and 800 vibrations per trillionth of a second. The effects of light of this limited range of frequencies are recorded in consciousness as different colors, a fact which we indicate by speaking, for example, of red light or green light. Though our recognition of frequency or color differences is a very important aspect of the relation between radiation and vision, I shall refrain from considering it further at the present moment. So much has already been written of color vision that it may well be ignored for once. Instead, we shall confine ourselves to the other way in which radiation may vary, that is, to intensity.

The effects of the intensity factor in radiation are recorded in our consciousness as brightness. The power

[1] This paper was presented under the title "Vision and Radiation" at a Symposium on Radiation and Life held by the American Society of Naturalists at its meeting at Des Moines, Iowa, on January 1, 1930. The interested reader will find the full details of the experiments here reported as well as all the references to the literature in two papers in the *Journal of General Physiology:* the first in volume 11, page 255, 1928; the second in volume 12, page 727, 1929.

to distinguish differences in the brightness of objects is an outstanding property of human vision, and is used constantly by us in judgments of distance and of form. The precise way in which this intensity evaluation is accomplished is only slowly becoming understood. In order to place before you the problem of intensity recognition and the possible nature of its basis in the eye, I can do no better than to deal with the relation of intensity to that function of the eye which is called its visual acuity.

Visual acuity, or the capacity of the eye to resolve the details of its environment, is capable of precise measurement in a variety of ways. The simplest method is by the use of Snellen's chart familiar from the oculist's window. The measurements consist essentially in determining the angular distance which must separate two contours so that the eye may recognize them as discrete; visual acuity is defined as the reciprocal of this angular distance. When this just discriminable distance subtends a visual angle of one minute, the resulting visual acuity is unity.

It has been known from the earliest times that visual acuity varies with the intensity of illumination. In daily life this describes the fact that at low illuminations we can not read the fine print which is easily legible at higher illuminations. The precise relation between visual acuity and illumination was first investigated by the astronomer, Tobias Mayer, in 1754 and since his time by many other people. The best of this early work is by Uhthoff, who studied not only white but colored lights at various illuminations. A few years later (1897) Koenig redetermined the influence of illumination on visual acuity in such detail that his data have become classic. They are reproduced in Fig. 1. All the experiments since then have only served to confirm Koenig's data. It is apparent from Fig. 1 that visual acuity varies in a specific way with the logarithm of the intensity of illumination.

In spite of the familiarity of this information and the existence of its precise description for about thirty years, there has appeared until recently no quantitative explanation of this relationship. Nevertheless the data create an extraordinary situation when one tries to realize their implications, and it is with these implications that we shall now be concerned.

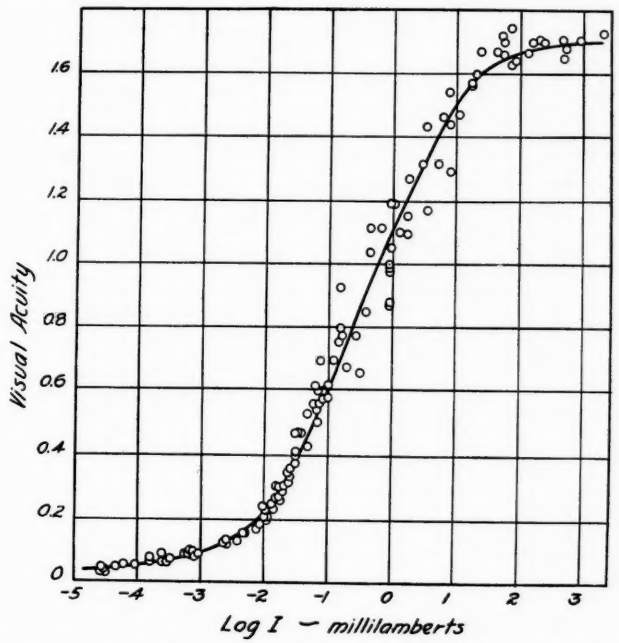

FIG. 1. Relation between visual acuity and illumination in the human eye. The data are those of Koenig. The curve is a theoretical one derived as explained in the text.

II

The fineness of detail which a surface, such as, for example, the photographic plate, can register depends on the number of receiving elements present in a unit area of the surface. In other words, the resolving power of a surface composed of discrete, independently functioning

elements varies inversely with the distance between the sensitive elements.

Consider what this means in relation to the data of visual acuity. The retina is a surface of this kind since it is composed of discrete rods and cones which function as units or as groups of units. Visual acuity measures the resolving power of this retinal surface, and the way in which visual acuity varies with illumination indicates the way in which the resolving power of the retina varies. A low visual acuity means that the average distance between the retinal elements is large, whereas a high visual acuity means that the distance is small.

To account in such terms for the large variation of visual acuity with illumination one must suppose that the distance between the sensory elements in the retina can and does vary nearly one hundred fold with the illumination. The data of visual acuity must therefore mean that at low illuminations there are fewer rods and cones present in a given retinal area than at higher illuminations. But we know that this can not be correct anatomically, because the rods and cones are fixed in the retina. They do not separate and come together, nor do they increase or decrease in number. Therefore, since they do not vary in number structurally, we must assume that they vary in number functionally.

In order to accomplish this let it be supposed that the individual rods and the individual cones do not all possess the same sensitivity to light. Some are stimulated by low illumination, others only by higher illumination. Then at low illumination the resolving power of the retina and therefore visual acuity is low because there are fewer sensory elements functional in it. As the illumination increases and more and more retinal elements reach their threshold of stimulation, visual acuity continues to increase. Finally at the highest intensities all the elements are functional and no further increase in visual acuity can take place.

It is possible to make this hypothesis strictly quantitative so that it may be compared directly with the accurate numerical data of Koenig. Before this can be done, however, it is necessary to consider one variable which was not controlled in these measurements, namely, the size of the pupil. This also varies with illumination and, if ignored, gives an erroneous idea of the actual illuminations on the retina itself which result from the recorded illuminations outside.

A correction for this variable is possible in terms of the measurements of Reeves. Fig. 2 is computed from Reeves's data and gives the relation between pupil area and illumination. The outside illumination given in Fig. 1 multiplied by the pupil area in square millimeters as recorded in Fig. 2 gives the resulting illumination in

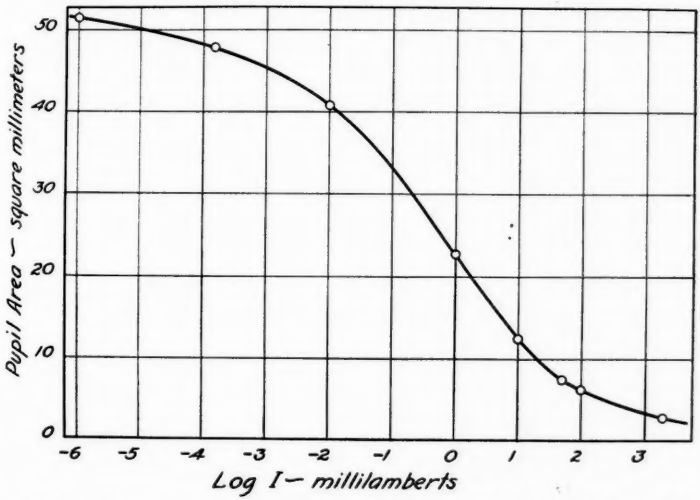

FIG. 2. Relation between pupil area and illumination calculated from the data of Reeves.

photons or units of retinal illumination. It is these corrected data which may be analyzed quantitatively.

III

There are several million rods and cones in the retina. In terms of a great deal of information already available and incorporated by von Kries as the duplicity theory, these elements may be considered as two distinct populations: one, the rod population, sensitive to low illuminations, and located in the retina outside of the fovea; and the other, the cone population, sensitive to higher illuminations, concerned with color, and situated all over the retina, but very densely in the fovea. If these two populations are like all other biological populations with which we are familiar from the work of statisticians, then their variation in sensitivity to light, which we have assumed to be the basis for visual acuity, should show the usual type of statistical distribution curve. Fig. 3 shows the frequency distributions for the thresholds of the rods and cones which have to be assumed in order to describe quantitatively the corrected data of Fig. 1. It is appar-

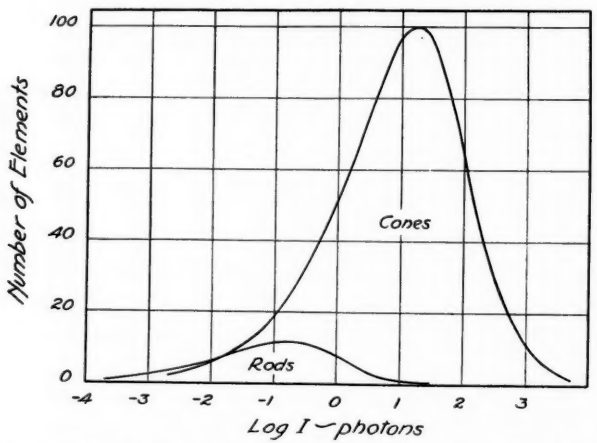

FIG. 3. Distribution of thresholds of rods and cones. The intensities are in photons, a unit introduced by Troland to represent the retinal illumination produced when the eye looks at a brightness of 1 millilambert through a pupil of 1 sq. mm. The two curves are identical in form, but different in position and in size of ordinates.

ent that these curves are similar to the usual frequency curves of the biometricians.

The curves in Fig. 3 are differential curves and give the number of elements whose specific thresholds correspond to a given intensity. What we wish to know, however, is the total number of rods and cones which are functional at a given intensity. These are given by the integral curves in Fig. 4. The integration of the curves of Fig. 3 to form those of Fig. 4 consists simply in finding at any intensity the area under the curves in Fig. 3 to the left

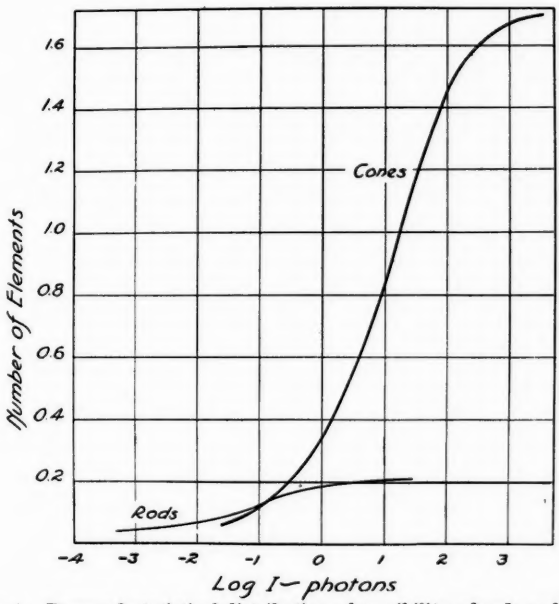

FIG. 4. Proposed statistical distribution of sensibility of rods and cones. These curves are the integrals of those in Fig. 3, and give the relative number of elements functional at any intensity. The ordinates read directly in units of visual acuity. The curves may be described by the common Gram series of the statisticians. However, the equation here used for them is $KI = x^2/(a-x)$ where K is a constant, I is the intensity, x is the corresponding visual acuity and a is the maximum visual acuity. K and a have different values for the two curves. This equation is derived from certain photochemical considerations for the details of which the reader is referred to the original papers in the *Journal of General Physiology*.

of that intensity. It is obvious that these two integral curves resemble the visual acuity data of Fig. 1, though of course it will be remembered that they represent them as corrected for pupil area.

Beginning with the lowest illuminations vision is a function of the rods. The number of rods which are active is very small. Since these active elements are spread out at random this amounts to having a resolving surface with the receiving elements sparsely distributed. The retinal distance between two just discriminable contours will be large and therefore visual acuity will be very low. As the illumination increases, more and more rods reach their thresholds and become functional; this results in a corresponding increase in visual acuity. Presently an illumination is reached when the first cones begin to function. Visual acuity will still be mediated by the rods because there are still more active rods present than cones. But as is apparent from Fig. 4 the rate at which the cones come into play with increased illumination is nearly ten times as great as the rate of the rods. Therefore at a certain point the number of cones functioning in the fovea will be equal to the number of rods in the retinal periphery. If the intensity is increased beyond this, the number of active foveal cones will be greater than the number of active peripheral rods in a similar unit area, and visual acuity will now be determined by the cones. This augmentation of the number of functional cones and the resulting rise in visual acuity will continue until all the cones are active and no further increase in visual acuity is possible.

The curves in Fig. 4 are corrected for pupil area. To show that they describe the original data of Koenig the continuous line in Fig. 1 is drawn. This line is derived from the curves in Fig. 4 and is transferred back to the original uncorrected data by means of the pupil area curve in Fig. 2. It is apparent that the theoretical treatment as represented by the curve in Fig. 1 serves as an

adequate description of the relation between visual acuity and illumination.

It may not be out of place to mention here that the integral distribution curves of Fig. 4 have more than a statistical background. Actually they have been derived from certain photochemical considerations which in themselves are of significance for the photosensory process as a whole. However, it is not possible to enter into this phase of the matter here.

IV

There are several implications from these data and from our analysis of them. I shall consider only one of these largely because of its historical and theoretical interest. The data heretofore given are for the normal eye, and the explanation proposed for them obviously rests on von Kries's theory of the functional separateness of rods and cones. Koenig recognized this when he attributed the lower limb of the data in Fig. 1 to the rods, and the remainder to the cones. He also recognized the implication involved, namely, that in a completely color-blind eye the cone portion of the curve should disappear and leave only the lower rod limb plus any extensions of it. Fig. 5 gives the data for two such individuals, one measured by Uhthoff and the other by Koenig. A comparison of these data with those in Fig. 1 bears out Koenig's supposition that the two limbs of the normal curve follow von Kries's duplicity theory.

These data are equally significant for our explanation. Fig. 4 shows that for the normal eye the cones overtake the rods at the middle of the rod range. It therefore follows that in a completely color-blind eye the visual acuity data should extend to a distance beyond the rod-cone intersection point equal to that which has preceded it. Moreover, the entire visual acuity data of such a case should be described by only the rod curve of Fig. 4.

The lines drawn in Fig. 5 are this rod curve taken from the theoretical curve of Fig. 4 and corrected for pupil

area as already explained. It is apparent that both our suppositions are correct. The data of the completely color-blind extend over twice the range of the rod vision of the normal. There is no rod-cone inflection point and the entire data are describable in terms of the rod distribution curve alone.

It must be apparent that in the complementary curve of a night blind person the rod portion of the curve should be lacking and the entire visual acuity curve should be describable by the cone curve alone. Such a case has not been studied in this way.

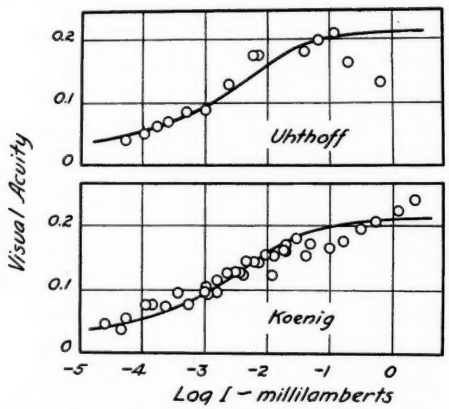

FIG. 5. Visual acuity of two completely color-blind individuals. Compare this figure with Figs. 1 and 4, but note that the ordinates here are for esthetic reasons made twice as large as in Fig. 1.

V

These experiments have been confined to the human eye. It must be clear, however, that the essential idea which underlies the theoretical treatment is of fundamental importance in an understanding of the effect of intensity on the visual process in its widest aspects. If the explanation of the dependence of visual acuity on illumination which we have proposed has any general validity in the physiology of vision; in other words, if the

number of receptor elements functional in a sense organ does vary with the intensity of the stimulating agent, then visual acuity should vary with illumination in other animals in a manner resembling that in man. It is important to recognize what we mean by the words "other animals." It is not to the point to repeat these experiments with other mammals, for example, or indeed with other vertebrates, because the eyes of all vertebrates are built on essentially the same plan.

In order really to test this idea, measurements must be undertaken with an animal whose eyes are built on a totally different plan from that of the vertebrate eye. Such a situation is found in insects, where the eyes are composed of ommatidia and there is no lens to form an image of the kind found in the vertebrate eye. Different as the eye of the insect is, it nevertheless possesses this in common with the vertebrate eye: it is composed of a series of independently functioning but related elements. Therefore if the theoretical treatment of visual acuity which I have given of the human eye is valid it should be applicable to the visual acuity of the insect eye. To determine this we undertook in our laboratory a series of studies on the visual acuity of insects and its relation to illumination. In the first of these Wolf and I investigated the visual acuity of the honey-bee. Recently Wald and I have been investigating the visual acuity of Drosophila.

The problem of studying the vision of animals other than man is made difficult by the impossibility of verbal communication between the animal and the investigator. It is obviously absurd to expect a bee to read Snellen's charts. We therefore had to develop a method for the investigation of the vision of animals other than man. We started with the common observation that most animals with eyes respond to a sudden movement in their visual field, and we converted this into a quantitative method of measuring visual acuity. The ideas involved are somewhat as follows. If the visual field of a sensitive

animal is made up of a pattern of dark and illuminated bars of equal size, the animal will respond to a displacement of this field only when it can distinguish the components of the pattern. In case the animal can not resolve the black and white bars the field will appear uniformly illuminated and displacement of the pattern will elicit no response. If visual acuity varies with illumination then the capacity to respond to these movements in the visual field will depend on illumination and on the size of the pattern. One can in this way determine the relation between the size of the bars in the visual pattern and the minimum illumination at which a movement of the pattern causes a response in the animal.

The honey-bee is very sensitive to such changes in its visual field and responds by a reflex, sidewise movement of the head and thorax. If the bee is crawling the response becomes evident by a sudden change in the direction of its progression which is opposite in sign to the movement in the environmental pattern. It is an extraordinary sight to watch the precision with which a bee changes its direction of creeping under the conditions of these experiments. If the pattern is moved, say to the left, the creeping bee swings sharply to the right through an angle which is easily 45° and may be much more, and continues creeping in the new direction. During a single crawl of perhaps ten centimeters we have frequently made a bee alter its direction right and left by moving the pattern left and right as many as four or five times in rapid succession.

The behavior of Drosophila under similar circumstances is even more dramatic. Drosophila confined in a long, narrow, rectangular, glass cell will crawl continuously from one end of the cell to the other. If the pattern of black and white bars which constitutes the visual field of the animal is moved in the same direction in which the animal is creeping steadily it will stop sharply, turn around and reverse its direction of creeping. By moving the bars back and forth a Drosophila may be made to

change its direction of creeping over and over again within a space of about 1 or 2 cms.[2]

Using this type of technique we prepared a series of plates composed of equally wide opaque and translucent bars, each plate having a different size of bar. Our experiments then consisted in determining for each size of pattern the minimum illumination at which a bee or a Drosophila would just respond to a movement of that pattern. The reciprocal of the visual angle subtended by each size of bar is then the visual acuity of the eye at the corresponding illumination. Fig. 6 shows our results

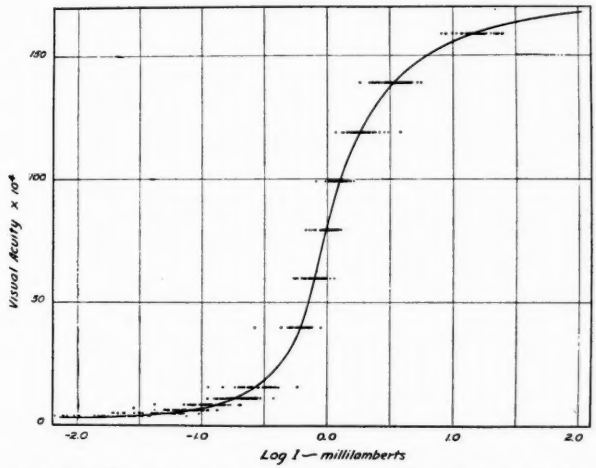

FIG. 6. Relation between visual acuity and illumination in the bee. Each dot represents a single measurement with a single bee. The curve is drawn through the mean values of the measurements.

secured with ninety-one normal bees. An effort has been made to represent with a dot each measurement with each bee. This is manifestly impossible in the places where the measurements come very close together. The plot therefore shows the general way in which the measure-

[2] At this point a motion picture was shown demonstrating the response of Drosophila to a moving pattern of black and white bars such as is used in the measurements here recorded.

ments run. It is apparent at once that the visual acuity of the bee's eye is related to the intensity of the illumination very much as is the case for the human eye. The results with Drosophila are essentially similar.

Certain comparisons may be made with these three animals. The maximum visual acuity for the human eye is between 1.5 and 2. The bee's maximum is 0.017. In other words, we can resolve the environment about one hundred times better than a bee can. The maximum visual acuity of Drosophila is 0.0015, which is only about one thousandth of our visual capacity. Moreover, our lowest visual acuity is about 0.03. Incredible as it may therefore seem, the greatest capacity for the optical resolution of its environment of the bee and of Drosophila is never better than ours is at our worst. If we recall our ability to discriminate objects in the dimmest surroundings we can get some idea of the maximum performance of which the insect eye is capable. This of course does not refer to the brightness which the animal may associate with the surroundings but only to the resulting visual acuity.

VI

In spite of these differences in magnitude between the visual acuity of the human eye and that of the insect eye the relation between visual acuity and illumination as shown by Fig. 6 is essentially the same in the two. The resolving power of the insect eye is low at low illuminations and increases with the logarithm of the illumination up to a maximum in the same sigmoid way as the human eye. The same kind of theoretical treatment is therefore indicated.

Differences in resolving power such as are found here must mean differences in the angular distances which separate the centers of the receiving elements. Since obviously the ommatidia can not vary their position under illumination, the data must be interpreted in such a way as to secure a functional separation of elements which

are structurally fixed. The solution to this problem we have already had. We may suppose that the receptor elements in the ocular mosaic do not all possess the same threshold but that the threshold varies among the ommatidia as does any other characteristic in a population. This then works out so that at low illuminations only a few ommatidia are functional; as the illumination increases, more and more become functional until an illumination is reached which is above the threshold of the most insensitive ommatidium. Visual acuity obviously runs parallel to this behavior of the ommatidia.

Before formulating this explanation in strictly quantitative terms it is necessary to examine more closely the nature of the data in relation to the structure of the eye. In these experiments the visual pattern was so arranged that it registered horizontally across the long axis of the bee's eye. At an illumination when all the elements are functional the maximum visual acuity then occurs when a horizontal row of elements receives light and an adjacent row receives no light, and so on. The size of the smallest perceptible pattern must in this way correspond to the visual angle which separates the centers of two adjacent ommatidia. The measurements as plotted in Fig. 6 show that the maximum visual acuity of which the bee is capable at the highest illuminations is about 0.017. This corresponds to a visual angle of between 0.9° and and 1.0°.

Since at these illuminations all the ommatidia are functional this experimentally determined, minimal, angular separation should correspond to the lowest vertical separation between adjacent ommatidia as determined anatomically. At the time when our experiments were nearly completed there appeared a study by Baumgärtner of the histological structure of the bee's eye in which he measured the angular separation of adjacent ommatidia in different sections of the eye. Fig. 7 reproduces his material. In vertical section the angular separation increases about four times from center to periphery. The

smallest separation is near the middle in the lower half of the eye and includes about twenty-five elements in vertical section. Its value lies between 0.9° and 1° and corresponds precisely with what Wolf and I found experimentally.

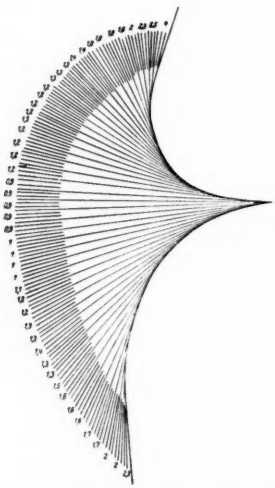

FIG. 7. Vertical section of the bee's eye, showing the way in which the angular separation of adjacent ommatidia varies in different parts of the eye. The ommatidia are drawn in groups of three. The drawing is reproduced from the work of Baumgärtner.

VII

One of the great advantages of working with the insect eye is the possibility of making experimental modifications in its surface. Fig. 7 shows that the angular separation of adjacent ommatidia increases from about 1° at the center to about 4° at the periphery. The increase is gradual, the middle half of the eye constituting a region of small angular separation in comparison with the rest of the eye—a sort of fovea. What should happen if this region of small angular separation were rendered functionless? Clearly the maximum visual acuity should be decreased since it would have to be mediated by

a region of the eye in which the functional elements have a greater angular separation. Moreover, the whole relation between visual acuity and illumination should become depressed in that the visual acuity at any illumination should be lower than normal and dependent on the area of the eye which has been rendered non-functional.

We made experiments in which a spot of black paint was placed in the center of each eye. It is difficult to place such a patch of paint with very great accuracy and to do it uniformly from eye to eye. We tried to cover about a quarter of the area of the eye. In vertical section such a patch of paint eliminates those ommatidia whose angular separation is less than about 1.3°. Since the maximum angular separation for the normal bee is between 0.9° and 1°, the maximum visual acuity of the bees with the centers of the eyes painted out should be between three fourths and two thirds of normal at the highest illuminations.

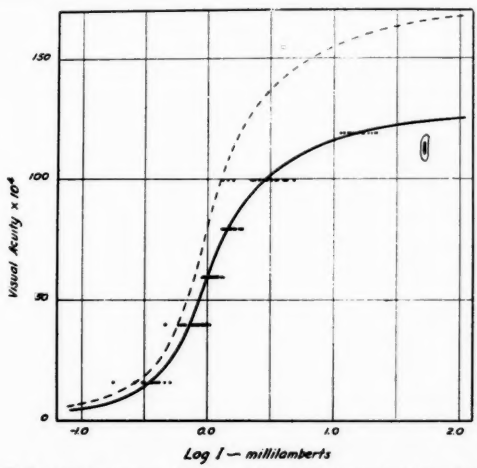

FIG. 8. Relation between visual acuity and illumination for bees with the central part of the eye painted out as shown in the figure. The points are individual measurements. The broken curve is the normal relation taken from Fig. 6, whereas the full curve is made from the normal one by multiplying its ordinates by 0.75.

We measured the relation between visual acuity and illumination in twenty-one bees whose eyes were painted in this way. The data secured with these animals are given in Fig. 8. The broken line is the normal visual acuity relation; the continuous line is drawn so that its ordinates are three fourths of the normal. It obviously describes the data.

In general the experiments which we have just described indicate quite clearly that when the total number of elements is reduced, the total number functional at any illumination is correspondingly reduced and therefore visual acuity as a whole is decreased. This may be shown in a different way by further experiments in the painting of the bee's eye.

At any illumination there is undoubtedly required a certain number of elements for the reception of the stimulus pattern. Since the eye is very nearly symmetrical in its two halves, there will be a symmetrical distribution of these functional elements on either side of the central vertical axis. If now one side were rendered functionless this should at once reduce the number of active elements by about half. To get the same number of elements as before in order to resolve the pattern, elements which are more widely separated would have to be drawn on and at once the visual acuity would be decreased. For example, suppose that for the eye to perceive the pattern as a series of bars, each bar would have to stimulate at least two functional ommatidia. These two ommatidia would most likely be distributed on either side of the eye. If one side is made non-functional this would reduce the number of functional ommatidia to about half. Then in order to have two elements determine a bar as before, the width of the bar would have to be doubled and the resulting visual acuity would be about halved.

We made measurements with nineteen bees in which the anterior half of each eye was painted out. The results are given in Fig. 9, in which, as before, each dot

represents a single measurement with a single bee. In Fig. 9 the broken line is the normal curve while the full line through the data is this normal curve with its ordinates multiplied by 0.62. It is therefore apparent that the reduction of the total number of available elements in the eye of the bee reduces the visual acuity of which it is capable.

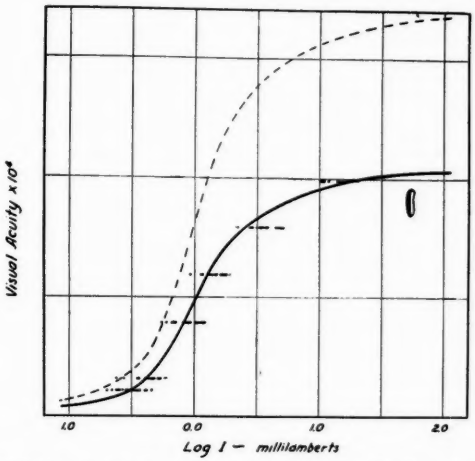

FIG. 9. Relation between visual acuity and illumination for bees with the anterior half of the eyes painted out. The points are individual measurements. The broken curve is the normal curve of Fig. 6, and the full curve is constructed from the normal by multiplying its ordinates by 0.62.

VIII

The experiments with the bees were so arranged that the black and white bars were received perpendicular to the long axis of the eye, that is, parallel to the long axis of the body. This longitudinal use of the bee's eye is not fortuitous. As is shown in the insets in Figs. 8 and 9, the eye of the bee is about four times as long as it is wide. Furthermore, according to Baumgärtner's measurements, the angular separation between adjacent ommatidia is more than three times as great in the horizontal meridian as in the vertical meridian. Both these

facts tend to make the bee's eye an organ which functions essentially as a vertical, linear receptor.

We found this to be true experimentally because Wolf and I were unable to get any response to a pattern arranged to register as bars parallel to the long axis of the eye. This is not a product of laboratory conditions because Baumgärtner also found that bees on the wing in the field are very astigmatic and resolve their environment vertically with much greater accuracy than horizontally.

It would follow from this that an eye which is more circular in outline should not suffer from the same difficulty. This is borne out by our experiments with Drosophila. It will be remembered that the eye of Drosophila is practically circular in outline. Wald and I have found that Drosophila will respond to a moving pattern regardless of whether this pattern is vertical or horizontal.

IX

Let us now return to the original relation of visual acuity to illumination as illustrated by Fig. 6. We have interpreted the shape of the curve in this figure to mean that the number of elements which are functional varies with the illumination. If the ocular mosaic were uniform it would follow that since visual acuity in the bee is determined by the vertical angular distance between elements the curve in Fig. 6 represents the number of functional elements in the vertical axis corresponding to any illumination. However, Baumgärtner's study as shown in Fig. 7 clearly demonstrates that the angular separation between adjacent ommatidia is not constant. The precise way in which it varies must therefore be considered in the transformation from visual acuity data to a statement of the number of ommatidia functional in the eye at any illumination.

Fortunately this is possible because of Baumgärtner's careful, anatomical study. From Fig. 7 one may de-

termine the actual number of ommatidia which are included in vertical section in any visual angle. Using that portion of the eye which contains the elements of smallest angular separation I have constructed a chart in which the angle occupied by each ommatidium is laid out on a linear scale of visual angles. This is not difficult because there are only about seventy ommatidia in vertical sec-

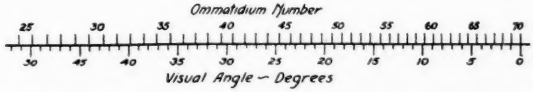

FIG. 10. Graphic representation of the number of ommatidia included in a given visual angle. The lower scale is a linear scale of visual angles. The upper scale gives the angle subtended by the individual ommatidia beginning with No. 70 at zero angle. No. 1 is the lowest ommatidium in Fig. 7.

tion in that part of the eye which is used in these experiments. Fig. 10 gives this relationship. From it one may compute the actual number of ommatidia in vertical

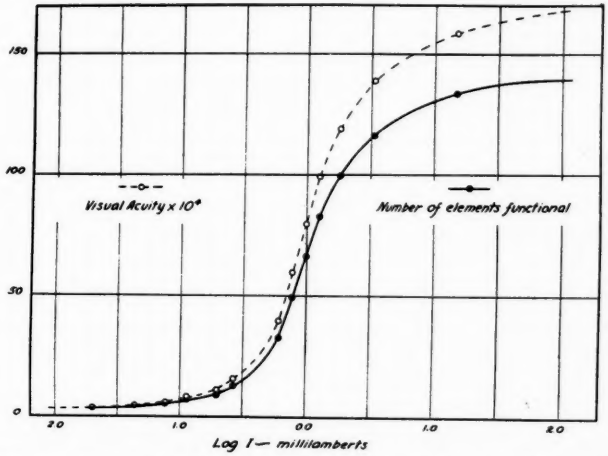

FIG. 11. Comparison between visual acuity and number of ommatidia functional in a given angular distance in their relation to the logarithm of the illumination. The ordinates of the number curve have been multiplied by 3.5 so as to make the lowest points of visual acuity and number coincide. The shape of the number curve is obviously that of an integral distribution curve.

projection which must be functional in order to furnish a given visual acuity.

The results of this computation are to be found in Fig. 11, where the number of elements in vertical section corresponding to a given visual acuity are given as well as the visual acuity itself. The curve describing the number of elements is not very different from the actual visual acuity curve. It resembles the usual integral distribution curves of the statisticians, even as its first differential the threshold curve given in Fig. 12 resembles the more commonly encountered differential distribution curves.

Therefore we may make our hypothesis of the relation of visual acuity and illumination in the bee quantitatively specific by stating it as follows. Taking the structural relations of the ocular mosaic as given by Baumgärtner, our data relating visual acuity and illumination may be described with complete fidelity by assuming a distribution of the threshold of the various ommatidia corresponding to the population curve of Fig. 12.

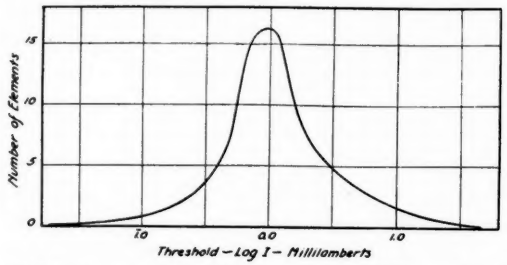

FIG. 12. Distribution of thresholds of the ommatidia in the bee's eye. The curve is the first differential of the number curve in Fig. 11.

X

The relationships between visual acuity and illumination which I have presented here are in themselves interesting and theoretically significant. They are, however, important in a still wider sense. At the beginning of this paper I pointed out that the variation in the in-

tensity of light is of considerable importance to vision. However, intensity recognition and intensity discrimination are by no means confined to the eye. Every sense organ discriminates intensities. More than that, nearly every organ in the body is concerned with evaluating intensity in one way or another. Does the information which we have gained from a study of visual acuity help us in any way to understand the problem which has puzzled biologists for many years, namely, how intensity recognition and discrimination are mediated by an organ? I believe it does. Indeed, it was for the purpose of shedding light on this particular problem that our investigations of the different aspects of visual acuity were undertaken.

It must be obvious—to confine ourselves to light—that an increase in the quantity of stimulating agent must produce an increase in concentration of the products of chemical or photochemical action in the sense cell. A naive way would be to suppose that a greater concentration of such products in the sensory cell produces a greater chemical or electrical effect on the associated nerve fiber which is then passed on to the proper center to find its expression in a sensation or in an affector like a muscle or a gland.

We know, however, that neither nerve nor muscle, nor, indeed, sense organs behave in such a simple manner. It has been repeatedly shown by Adrian, by Forbes and by others that the impulse along a single nerve fiber is constant in magnitude regardless of the size of the stimulus. The work of Lucas and others has demonstrated that the contraction of a single muscle fiber, with perhaps some exceptions, is of constant magnitude regardless of the stimulus. Moreover, Adrian's work has shown very much the same thing for the discharges from single sense cells. Nerves and muscles behave like quanta of energy in physics; they are either all or nothing. Just as the naive conception of intensity has disappeared in the physics of radiation and has been replaced by the idea of

number, so indeed is it disappearing in biology. A light that is more intense than another is merely emitting more quanta per unit time and space than another. Can we apply similar concepts to the understanding of the perception of intensity in biological systems?

There are two ways in which such ideas may be applied, and both of them have been suggested. The oldest is that of Lucas and was considered for muscle. Lucas had found experimentally that as the stimulus to a muscle is increased gradually its height of contraction increases in a series of discrete steps the number of which corresponds with the total number of individual muscle fibers present in the muscle. Intensity in muscle is therefore registered by the number of elements in the muscle which are functional.

When it was shown that the nerve impulse is all-or-nothing as well, another idea was suggested by Forbes. This is that intensity is determined by the number of impulses which proceed over a single nerve fiber in a given time; in other words, that the frequency of impulses in a nerve determines intensity. In fact, the beautiful researches of Adrian and his colleagues have shown beyond a doubt that the frequency in a single nerve fiber actually does increase with intensity.

Which of these two aspects of number applies to the intensity recognition of the visual process? Is it the number of unit structures or is it the number of impulses in a single structure per unit time? One thing is certain: whether we give it first place in vision or not, frequency is a factor in intensity transmission. This is true, as Adrian's work with the eel's eye has shown experimentally. The problem really is, first, whether the number of elements functional enters at all as a factor; and if it does, second, how the conception of frequency of impulses in a single element and the conception of number of elements work together to give intensity discrimination and perception.

On the face of it, it is difficult to consider frequency alone as the basis of intensity discrimination. Frequency is a continuously variable function because time is continuous. But it is a fundamental property of intensity recognition that it is discontinuous in vision as well as in muscle. For example, Koenig found that over the whole range of intensity, that is, about ten million units, within which the eye is capable of functioning, the eye can recognize with certainty only about 572 distinct steps in intensity. This discreteness of intensity discrimination is characteristic of all sense organs which have been investigated in this way. How to describe it in terms of a continuous function like frequency does not seem apparent. It is therefore necessary to turn to the idea involving number of elements functional.

XI

The results of our work, as you are already aware, constitute independent evidence of the fact that differences in intensity are associated with differences in the number of elements functional in the retina. Visual acuity therefore answers our intensity question at once. However, the relation between the two phenomena, that is, between visual acuity and intensity discrimination, needs to be considered in further detail before we can be satisfied with this answer. Of the 572 discrete steps in intensity which Koenig was able to find for the eye, the first 30 are mediated by the rods; the remainder, 542, by the cones. If the recognition of intensity differences is connected with a change in the number of elements which are functional, then a minimal intensity difference must mean a change from n to $n+1$ or $n-1$ in the number of cones functional in a unit retinal area. Consequently a minimal retinal area in the fovea, where the cones are, must contain 542 cones in order that it may be able to discriminate 542 steps. Only in this way can each step in intensity discrimination correspond to an

increase or a decrease in the number of functional cones in a unit area.

If visual acuity and intensity discrimination are manifestations of the same mechanism, such a minimal retinal area should also be able to mediate all the visual acuities, from the lowest to the highest. Such an area will have for its linear dimensions the retinal distance between two just perceptible contours corresponding to the lowest visual acuity. The retinal distance for all other visual acuities will obviously fall within this distance. From Koenig's data the lowest visual acuity is very nearly 0.03 units. This corresponds to a visual angle of 44 minutes and to a retinal distance of 0.2 mm. The resulting square area is 0.04 sq. mm. The fovea contains about 13,500 cones per sq. mm. This minimal visual acuity area of 0.04 sq. mm therefore contains 540 cones, which is the same number as that derived from the number of steps in intensity discrimination. It would therefore seem that the increase or decrease in the number of elements functional takes care of visual acuity as well as of intensity discrimination.

What then becomes of the frequency of discharge of a given cell? This surely exists in the retina and undoubtedly has a function. I believe that not only can we fit it into the scheme but that it is really essential to our notions if the story of intensity discrimination is to be complete. I have already emphasized the fact that intensity recognition and intensity discrimination are discontinuous functions. Let us place intensity I on one side of a photometric field and the just next perceptible intensity $I + \Delta I$ on the other side of the field. According to our ideas, I corresponds to n cones functional per unit area and $I + \Delta I$ to $n + 1$ cones. What happens if we were to put an intensity midway between these two, say $I + \Delta I/2$, on one side of the field? How many cones would this correspond to? Obviously to n again. Now what is the next just perceptible intensity to this? Experiment shows that it certainly is not $I + \Delta I$, though this presumably corresponds to the $n + 1$ cones. The

next perceptibly different intensity is very nearly $I + \Delta I/2 + \Delta I$. This seems absurd since if $I + \Delta I/2$ corresponds to n cones, then all that one should need to go to is $I + \Delta I$ previously known to correspond to $n + 1$ cones. In other words, the step ΔI is a specific magnitude for each individual intensity I. Changing the intensity merely changes the required ΔI to the value corresponding to that particular intensity. There is thus always a just discriminable step in intensity recognition no matter from which intensity one starts. Intensity discrimination possesses no singular points.

The solution of this paradox lies in a recognition of frequency as a factor in intensity perception. Take any given sense cell with its specific threshold. It will begin to function at its proper intensity threshold and will then discharge at regular intervals so long as light of that intensity continues to shine on it. As the intensity increases the frequency of its discharge will increase steadily. However, presently the threshold of the next most sensitive cell will be reached. This will then begin to function at its minimal frequency which will also increase with intensity. Since we have found that intensity discrimination corresponds to a definite increase in intensity, this must correspond to an addition of an element functioning at the same frequency as the last one. In other words, intensity discrimination in this analysis depends on a definite increase in the frequency of discharge of a series of related retinal elements, this increase being made by the addition of a new functional element.

In this way there may be harmonized the two characteristics of intensity recognition: first, that intensity discrimination is discontinuous; and second, that there are no critical points in intensity recognition. By the same token there are combined in the explanation of these two paradoxical characters the two known happenings in the sense organ, namely, a variation in the number of elements functional and a variation in the frequency with which each element functions.

RADIATION AND GENETICS[1]

PROFESSOR H. J. MULLER

UNIVERSITY OF TEXAS

I. THE EFFECTIVENESS OF THE AGENT

WHEN a geneticist speaks of radiation, he usually refers only to that portion of the spectrum having a wave-length at least as short as that of X-rays. Altenburg has shown that even ultra-violet light has little if any effect upon the genes comparable with that of radiation of higher frequencies. On the other hand, the studies of Hanson and of Stadler have proved that increasing the frequency of the rays beyond that of X-rays does not deprive them of their genetic effectiveness, inasmuch as the gamma rays of radium work just as well. So do the beta rays, or electrons themselves, as Hanson has shown. This is, of course, as he has pointed out, just what we should expect if the X-ray effect is a direct one, dependent almost entirely on (the disruptions caused by) the released electrons; in that case "cosmic" rays must necessarily act likewise.

I do not wish to review in detail here the work of the years 1926 to 1928, in which it was shown that high-frequency radiation, such as releases electrons, does produce, in abundance, heritable changes in animals and plants. The positive evidence, not only from our laboratory, but also that of Stadler, of Weinstein, of Hanson and his colleagues, of Goodspeed and Olson, of the Whitings, of Blakeslee and his coworkers, of the Timofeëf-Ressovskys, of Serebrovsky and his colleagues, of Dobzhansky, of Grüneberg[2] and of others admits of no

[1] Paper read before the American Society of Naturalists at the Des Moines meeting, January 1, 1930, in symposium on "Radiation and Life." A few additions have been made to bring the treatment up to date (February 8, 1930).

[2] The work of the last two investigators named was first reported in 1929.

doubt. It is also clear that these heritable changes are of two main kinds: changes in what I may call the gross morphology of the chromosomes, and ultra-microscopic changes of the sort that have been termed "point mutations" or "gene mutations."

II. Evidence for the Production of Mutations that are Not Destructive

Granted these facts, however, it still remains for us to consider what the significance of these effects may be, how the changes are produced and what light a study of the changed germ-plasms themselves may throw on the properties of the genetic material. Can it be that we have in the X-ray tube and in the radium needle merely an amusing but not very instructive toy, wherewith to produce all sorts of bizarre monstrosities that are very pretty for us to play with, but that, after all, these "laboratory deformities" can have but little bearing on the constructive evolutionary processes of organic nature and on their physical basis? It has been hinted in more than one place that all we have done is to break and knock holes in the chromosomes, perhaps, too, to get the pieces tangled together sometimes, but that such stunts can no more help us in a real understanding of our object than would the wrecking of a train help us to understand the normal workings of a locomotive. Are we simply wreckers? How can we meet such criticism?

The principal argument for the claim that even the so-called point mutations are really just the more minute of the "chromosome abnormalities" lies in the fact above mentioned that both are produced by the same agent—radiation. In this connection, I may point in the first place to evidence which I have gradually accumulated, indicating that the production of point mutations is to some extent separable from that of chromosome abnormalities, or at least from some kinds of chromosome abnormalities. A study of the data derived from irradiation of

mature females and mature males shows consistently that in the sperm the frequency of displacements of chromosome segments, as judged by the phenomenon of crossover-reducers, is raised much more, in comparison with the frequency of such changes produced in females, than is the frequency of "point mutations." That is: both effects—"point mutations" and "displacements"—are produced in both sexes; both effects are produced in considerably greater abundance in the sperm than in the oocytes, with a given dose; but the ratio of the displacements to the point mutations is higher in the treated sperm than in the similarly treated female germ-cells. Those who argue that the two effects (chromosome abnormalities and point mutations) must be alike because their production is caused by the same agent (X-rays), would now surely have to reason, correspondingly, that the two effects are not alike because their production is affected differently by another agent (sex), and so the whole original contention loses what pertinence it may have had.

Most cogent is the evidence from reverse mutations. Hanson, in 1927–28, found several reverse mutations of bar eye to round after X-raying, though none from round to bar appeared. Forked bristles have appeared at various times in our X-ray work. Since I reported the finding of a reverse mutation from non-forked to forked, Dr. Patterson has carried on experiments on a large scale, in cooperation with me, and the total results show eight mutations in the direction forked to non-forked and eight in the direction non-forked to forked, in the X-rayed series, and none in a comparable number of controls. Most of the mutations from non-forked to forked occurred in treated non-forked genes that had themselves arisen under X-ray treatment by mutation from forked to non-forked, so that the induced mutations are clearly reversible. These mutations were shown, by tests, to be due to changes at the locus of forked itself,

not to the origination of modifying or duplicate factors—"suppressors" or "mimics." Similar but less extensive findings have been made in the case of the locus of the so-called "scute" bristle condition. In the meantime, Timofeëf-Ressovsky, in Berlin, has also obtained, by irradiation, some dozens of different reverse mutations which, in his experiments, were scattered among different loci. It is quite impossible to reconcile these definite results with the view that all mutations are losses. For if, with one blow, we "punched the gene out," then with the next we punched it in again. In other words, if with one shot we wrecked the train, with another we unwrecked it, and just as easily. Moreover, if we suppose one of these mutations to be a "gain," due to the attachment of a chromatin segment from some other place in the chromatin, it is unreasonable, in the light of our knowledge of displacements, to suppose that this extra piece would time after time become attached at this same locus. Thus the evidence from the reversibility of the point mutations shows decisively that they are not mere losses and makes it very probable that they are not caused by chromatin displacements at all.

It may be repeated here that the "point mutations" arising after irradiation of male or female, adult or larva, in somatic or germinal tissue, are in no discoverable way different from those of apparently spontaneous origin which have previously been dealt with in the *Drosophila* work. So far as I have been able to ascertain, there is no greater proportion of lethals, as compared with visible mutations, among the X-ray "point mutations." There seems to be about the usual preponderance, among the usable X-ray mutations, of recessives as compared with dominants, and it is found that various allelomorphs are producible at a given locus. Thus, at the locus of white eye, four allelomorphs, apricot, eosin, a tinged-like allelomorph and white have already been produced in experiments of Patterson's and

my own,[3] although white occurs by far the most frequently, as it does in the case of the spontaneous mutations at this locus. We have found white in germinal tissue over a dozen times, following irradiation, and each of the other three allelomorphs just once. And not only the character effects but also the genetic behavior of the induced mutations are quite like those of the spontaneous ones. If the so-called "spontaneous" gene-mutations serve as the basis for evolution—and no other major basis has yet been discovered, despite the search of biologists on a grand scale—then the artificially produced mutations likewise must include amongst them artificial building-blocks of evolution as good as the natural stones. This conclusion is greatly strengthened by the establishment of the reversibility of the induced mutations.

However, our being able to imitate the natural articles does not necessarily imply that nature made them by the same method that we did, even though such a possibility is at once suggested. The determination of whether or not the mutations in nature are actually caused by the radiation in nature involves us in further experiments and calculations. Connected with these is the investigation of the way in which the X-ray effect itself is produced, a question which is of considerable interest because of its relation to some other problems also.

III. Studies of the Way in which the Mutations are Produced by Radiation

If the change in a gene is not a direct effect, caused by the tremendous concentrated energy of a local electron "hit," but is produced indirectly through the intermediary agency of injurious chemical substances or physical conditions that become diffused through the cell as a result of the irradiation of the latter, then it

[3] See also the very interesting studies by Timofeëf-Ressovsky on different allelomorphs induced at this locus, and the remarkable results of Dubinin on different allelomorphs induced at the locus of scute, by means of X-radiation.

becomes very likely that other chemical or physical treatments likewise will be able to produce mutations. If, on the other hand, the effect is solely a direct one (in the path of the electron), and no indirect mutational effect is produced by the rays, then, while it still remains possible that other physical or chemical influences also will be effective, it becomes likely that such influences would usually have to be rather specialized. For the chemical and physical changes produced by the X-rays themselves on the complex protoplasmic medium (other than on the genes directly) must necessarily be very varied, and, in the case of the heavy doses we use, some of these changes must be exceedingly drastic as well. This is shown by the non-genetic lethal effects of our X-ray treatments on many of the treated eggs and larvae. I say "non-genetic," because if the effects in question were genetic, the treated male larvae, bearing only one X, would be killed off in distinctly greater numbers than the treated female larvae, which are protected by two X's, whereas Dr. Patterson's records show that there is no such distinct differential mortality of the two sexes when embryos are treated. The high mortality of the larvae is, therefore, largely non-genetic—"physiological." These drastic unfavorable physiological changes which the X-rays give rise to, then, might well be expected, secondarily, to produce mutations, if mutations can readily be produced by numerous influences other than direct irradiation itself.

While this question concerning the directness of the effect is by no means finally settled, we do already have data that have some bearing on it. The original X-ray experiment had shown only 5 mutations among 6,016 control chromosomes,[4] or fewer than 1 in 1,000; 122 among 783 chromosomes that had themselves been treated, or one in about 6½, and 6 among the 783 chromo-

[4] Chromosomes that were not themselves X-rayed in the preceding generation or associated (after fertilization) with chromosomes that were X-rayed in the preceding generation.

somes that had not themselves been directly treated but that had been in untreated germ-cells which fertilized the germ-cells that were treated. The latter rate, of nearly 1 in 100, was unexpectedly higher than the approximately 1 in 1,000 rate found in the chromosomes that were neither treated themselves nor mated to treated. Although the numbers were not of decisive size, for cases of such low rates, nevertheless they raised the suspicion that perhaps there was an indirect effect of the radiation upon the untreated chromosomes, caused by their association with the treated cytoplasm or with the treated chromosomes from the other parent. This questionable effect was provisionally entitled "transverse induction," and was made more plausible by the fact that three of the six mutations in question arose in paternal chromosomes that entered treated eggs, although the eggs were treated in fewer than one third of the cases and then with a comparatively light dose.

I have therefore followed up this lead, X-raying females (with an average dose of about "t6¼" or 1,780 r units)[5] and crossing them to untreated males, and then testing the offspring for lethals by the ordinary "C1B" method. Controls derived from the same source were carried along in parallel. There were two separate lethals among the 984 control chromosomes, and there was but one, a semilethal, among the 867 untreated chromosomes that were immersed in treated cytoplasm. I conclude therefore that the earlier figures probably represented the chance deviation common among small numbers, and that as yet there is no sound basis for a belief in "transverse induction."

[5] We find that the so-called "t 1" dose, with our present machine, corresponds to about 285 r units. The "t" doses in the data of the present paper were all given with this machine. The "t" doses referred to in my papers of 1927 and 1928 were, however, given with a different machine, and most of them were approximately three times as strong; *i.e.*, the old "t 1" usually was about 855 r units.

Experiments were also carried out to determine whether there was any delayed effect—"delayed induction." An altered physiological condition capable of producing mutations might persist for a while and continue to produce mutations for some time after treatment, but a direct local effect would be more likely to be immediate. A mutant gene arising at once in a treated gamete would be contained in all the cells of the resulting individual, or in half the cells if the chromosomes are already split in the gametes, but a mutant gene whose origination is delayed until some time in the cleavage stages or later will be present in a smaller patch of the adult somatic tissue. This will be a fairly coherent patch, however, as Bridges' and Stern's work with patches in the "Minute" stocks, Sturtevant's work on mosaics in crosses of "claret" females and Patterson's work on raying larval cells have shown.

For this experiment, normal males were given a heavy irradiation ("t 13," or about 3,700 r units) and crossed to yellow females with attached X's. The eyes of the sons, containing their father's X (treated), were carefully examined to find mutations to white among the facets. The more delayed such a mutation, the smaller the group of affected facets should be, but the more cells of that stage would have been present for the mutation to occur in, and therefore (other things being equal) the chances of finding the smaller patches would be proportionally greater. The experiment was done on such a scale that if the tendency for the delayed mutation to white, after some fifteen to twenty cell-divisions, had been reduced to only about one one-thousandth of its original tendency, still such a mutation, in a single facet or small group of facets, should have been found. The examination of facets was kindly carried out for me by Dr. Patterson, whose large amount of experience in this work enables him readily and unerringly to detect such changes even in individual facets.

Among 3,533 male flies carefully examined, including approximately six million observed facets, not one of the type sought for was found. No white facets at all were seen, except in a single fly, which had eight white facets, not coherent but scattered, four on each eye. This fly clearly did not represent a case in point, but rather a case of an eversporting condition, such as has been found on various other occasions, and the nature of which will be discussed later. We may conclude, then, that there is little if any tendency to "delayed induction." This, by the way, strengthens the conclusion which I had previously reached that the tendency for a mutation to appear in only half a fly derived from treated sperm, instead of in the whole fly, indicates not that the mutation is delayed to the two-nucleus stage of cleavage but rather that the chromosome may be split already in the mature spermatozoon.

In further investigation of the manner in which the X-ray effect is produced, experiments were undertaken to study the possible influence of various external and internal factors acting along with the X-rays. In one experiment, two batches of flies of identical origin were irradiated heavily (t 12 ++) and simultaneously, but one was kept at a temperature of 34° C. and the other at 8° C. during the irradiation. Twenty-two of the 67 F_1 cultures in the cold series, or 33 per cent., showed lethals or semilethals, and 32 of the 120 F_1 cultures in the warm series, or 27 per cent., showed such mutations, the difference being not significant. A repetition of this experiment on a larger scale gave similar results. In the second experiment a "t 8 +" (about 2,300 + r units) treatment was applied, and temperatures were maintained as before. The cold series comprised 403 cultures, containing 33 lethals and semilethals, or 8.2 per cent.; the "warm series" comprised 208 cultures, containing 13 lethals and semilethals, or 6.2 per cent. The intensity of radiation in both series of both experiments

was doubtless significantly greater than that calculated and given above (hence the "+" signs used), on account of the fact that the small gelatin capsules containing the flies lay in copper containers, which must have given off secondary radiation. In the capsule at the lower temperature the flies, anesthetized by the cold, lay motionless against the bottom of their capsule, and therefore remained at a smaller average distance from the copper than that of the active flies in the warm capsule. Possibly this is the cause of the somewhat higher mutation rate found in the cold series in both experiments. Certainly, however, we may conclude that there could have been no great *rise* in rate with increased temperature, such as would have occurred in the case of an ordinary chemical reaction, for a 26° C. rise in temperature raises the rate of an ordinary chemical reaction from six to sixteen times. Stadler has independently obtained similar results in experiments in which barley was subjected to different temperatures while being X-rayed.

Another factor whose influence was studied was metabolic rate. Anabolism and multiplication of oogonia and oocytes is more active in well-fed females than in starved ones, and in impregnated females than in virgins. Starved virgin females, when irradiated with a "t 4" dose (1,140 r) and later mated and fed, gave five lethals and two doubtful lethals in 219 F_1 cultures; fed virgins gave four lethals and one doubtful lethal and one visible in 298; fed impregnated females gave six lethals and two visibles in 260—again no decided or significant difference (though further work, yielding large numbers, will be desirable in view of the low induced mutation rate in females).

Again, it was thought that the genetic situation, *e.g.*, the arrangement of the genes, might possibly affect their susceptibility to being transmuted—especially since some preliminary tests on a rather small scale had seemed to indicate that a certain inverted X-chromosome produced

by X-rays, called "delta 49," in which a long section in the middle is turned around, gave fewer mutations. But in a more critical irradiation experiment in which 424 F_1 cultures from the inverted irradiated chromosome ("t 13" dose, or about 3,700 r) were examined, and 432 from normal chromosomes rayed likewise, 49 lethals, one doubtful lethal, one semilethal and four visibles were found in the inverted series, and 53 lethals, three doubtful lethals, four semilethals and six visibles in the non-inverted—virtually the same numbers.

On the other hand, there are internal conditions which do affect the frequency of mutations produced by radiation. Thus we have seen that the rate of induction in the mature sperm is higher than that in adult females. It is also higher in the mature sperm than in the larval males[6] (unless in the latter case a large selective factor enters in, to allow later a higher reduplication rate on the part of most of the non-mutated than of the mutated spermatogonia), and it is higher in the mature sperm than in the larval females. The former relation may be illustrated by citing an experiment (of April, 1928) in which larval males from three to four days old, after having been reared at 27° C., were treated with a "t 8" dose (about 2,300 r units), and later bred to yellow females with attached X's. Among 2,651 F_1 males examined, only one visible sex-linked mutant was found (this one contained two visible mutations in its X chromosome, at separated loci!), and only one male showing an autosomal dominant mutation appeared. Other work of the author has shown that in a count of this size derived from males given this amount of irradiation when adult, and bred soon afterwards, there would be, on the average, about seven visible sex-linked mutations and a comparable number of autosomal dominants (see Table I). Similarly, Harris and Hanson independently

[6] This seems not to be true in the case of forked reverse mutations, however.

TABLE I

FREQUENCY OF VISIBLE MUTATIONS IN GERM-CELLS OF IRRADIATED MALES CROSSED TO UNTREATED FEMALES WITH ATTACHED X'S

Stage at which P_1 male was X-rayed	Dosage	Number of F_1 males counted	Calculated equivalent number for "t 8" dose	Number of visibly abnormal F_1 males	Number of visibly abnormal F_1 males able to breed and transmit their abnormality, in:		Nature of X in treated flies	Date of experiment
					X	autosome		
Adult	t 16	620	1,240	52	4	13	"δ 49" bobbed	Nov., '28
"	t 8	1,724	1,724	59	8	7	bar	Jan., '29
"		2,344	2,964	111	12	20	Sum of above	
Larva 3–4 days old	t 8	2,651	2,651	20	1 (double)	1	"δ 49" bobbed	Apr., '28

(Remarks: The personal equation enters to a certain extent into the interpretation of the count from the rayed larvae, as about half of this count was made by the author and half was made for him by Dr. J. T. Patterson, but after all possible allowance is made on this score, the difference between the numbers observed in the experiments with larvae and with adults still remains significant. More reliance is to be placed on the figures for the mutations in the X than for the dominants in the autosomes, since the latter are usually nearer the border line of detectability and their recognition is subject to greater fluctuations. The general relation above recorded between the mutations in the X and the autosomal dominants was observed in a third experiment also, performed by the author in October, 1927. This is not quoted in the table because the dosage (supposedly "t 4" on the older machine) was not certain. In this experiment, among a total of 2,769 F_1 males from P_1 rayed adult males having normal X's crossed to double-X females, 149 visibly abnormal F_1 were found, and of these, six were fertile and transmitted a sex-linked mutation and ten an autosomal dominant. In contrast to this, there may be quoted a recent (November, 1929) experiment by four students of the author, in which adult "δ 49" bobbed males, after a "t 8" dose (2,280 r), were crossed to double-X females; 9,518 F_1 males were counted, of which seventeen transmitted a sex-linked and only eight an autosomal dominant mutation. These relatively lower numbers, especially for the dominants, illustrate the effect of personal equation above referred to.)

have found the rate of lethals from mature sperm to be five or more times higher than that from spermatogonia treated in the adult male.

In another experiment, of the present author, when normal females were bred that had been rayed while larvae at the stage and with the dosage above mentioned for the larval males, no transmissible visible abnormalities at all were found among their 774 daughters (allowing detection of dominants) or 781 sons (allowing detection of dominants and of sex-linked recessives). About nine visible mutations should have been found in all, here, if the visible (to this observer) mutation rate pre-

viously found for mature sperm had held. In this latter experiment it is to be noted that the low apparent rate can not so plausibly be explained as due to a differential reduplication of non-mutated as compared with mutated gonial cells, since in the female the presence of two X's would tend to protect the cells from such effects.[7] The same general consideration applies to an experiment reported by Stadler on barley, in which he finds the induced mutation rate in growing apical buds of sprouting seeds or seedlings to be approximately eight times as high as that in the resting seeds. It would seem to follow that, under certain conditions of an intracellular nature, a "struck" gene is more apt to be permanently changed, and under other conditions, when similarly struck, it retains or returns to its previous equilibrium-configuration.

Another probable expression of intracellular differences in susceptibility to the gene-transmuting effect of radiation is to be found in the not infrequent observation of cases of double or multiple mutation, in which two or more loci in the chromatin of a cell have become altered simultaneously. These cases would seem to occur too often to represent mere coincidences. I had at one time thought them to be an expression of the "striking" of two genes by the same speeding electron, but recent calculations which Mott-Smith and I have made show that, with the strength of X-ray treatment which we use, a nucleus would always be traversed by many electrons anyhow, so that the striking of any given electron at a certain locus would not materially raise the chance of another, non-neighboring locus in the same chromatin being struck. If, then, the effect in question turned out to be valid (beyond the bounds of coincidence), it would have to be explained as due to a special susceptibility

[7] Mr. G. Moore is now undertaking experiments at the University of Texas to determine more systematically and exactly the relation of sex, and age at time of raying, to the frequency of the induced mutations.

on the part of the chromatin of the affected cell, due to its peculiar physicochemical condition.

In view of the various observations recorded above on the modifiability, through "internal" causes, of the gene-mutation frequency existing in the presence of artificially applied radiation, it might be expected that various influences would be found which could affect the gene-mutation rate materially in the presence of only the amount of radiation naturally present. Such effects might or might not be due to variations in the susceptibility of the material to this natural radiation (depending upon whether radiation were a necessary cause of mutation or not). As a matter of fact, the "natural" mutation rate, like that artificially induced, was found in experiments of Altenburg and myself to be highly variable, but the influences which cause most of this natural variability are as yet unknown.

We reported on the probable effect of temperature in moderately raising the natural mutation rate, even as it does that of chemical reactions. Although this apparent effect of temperature will not account for most of the natural variation in mutability, it becomes important, because of the contrast between this result and that recorded on page 228 of the absence of a rise in mutability with increase of temperature when X-rays are applied. This apparent contradiction would argue for the temperature effect being an acceleration of some other process or processes, that produce mutation in a manner similar to, but independent of, radiation, and it would, conversely, argue for the conclusion that radiation, for its own part, produces mutations "of its own accord," similar to and yet not by "accelerating" those processes which produce mutations naturally. On account of these bearings of the matter, I am now repeating the trial of temperature in the absence of artificial irradiation, since the earlier results, from a statistical standpoint, do not

yet seem as conclusive as would be desired for the establishment of such far-reaching principles.

Controlled factors other than temperature, acting in the absence of artificial irradiation, have so far failed to give positive results. Among the experiments tried and already mentioned in an earlier paper were treatments, carried on over the entire life-cycle, with all-but-lethal concentrations of lead acetate (1 per cent. of the food), of arsenic (0.015 per cent.), manganese chloride (0.3 per cent. to 0.6½ per cent.) and of Janus green (0.25 per cent.). All these were quite negative except possibly the Janus green. In the latter case, among 519 F_1–F_2 cultures derived from 16 treated P_1 males crossed to "C1B," there were 5 mutations: 2 lethals and 3 visible semilethals. One of the 2 lethals had arisen in the early germ tract of the P_1 male, being present in 5 of the 50 F_1–F_2 cultures derived from him; two of the three different semilethals arose in sister cultures also (in a batch of 40).[8] There were 1 lethal and 1 semilethal in the 482 controls for all these experiments. I have recently repeated the Janus green experiments, with the following results: 1 lethal and 1 doubtful lethal among 539 cultures, the control series to this yielding 3 lethals and 1 doubtful lethal among 531 cultures. Taking both experiments with this agent together—7 or 8 mutations in the 1,058 Janus green and 6 or 7 in the 1,013 control cultures—the results are distinctly negative.

[8] In an experiment of the same series in which adult males were subjected to 36° C. for from 40 to 64 hours (as long as they could stand it) four different lethals were found among 494 F_1–F_2 cultures derived from the 22 treated P_1 males which bred. Though 14 of these 22 P_1 males had yielded between 20 and 40 F_1–F_2 cultures each, 3 of the 4 different lethals were found among the 39 cultures derived from a single one of these males. This result is of interest in connection with the possible tendency for simultaneous production of mutations by X-rays in given cells, mentioned on page 232. In the case of X-rays, however, there has been no apparent tendency in my experiments for a given individual, as a whole, to produce more mutations than other individuals of the same batch, provided all are bred before the mutated cells have a chance to multiply. Serebrovsky has recently reported finding this chance distribution of mutations among the P_1 individuals in his X-ray experiments.

During the past year, Guyer's experiment of injuring the eye, which Morgan recently applied to *Drosophila,* with results doubtfully indicating a production of visible mutations, has been repeated. I found it advisable here to use a new genetic method, which I call "Cs ♀ B" in contradistinction to the older "C1B" method, and which allowed the treatment to be applied to wild-type females. After being treated, they were mated to "Cs ♀ B" males. The latter contain a crossover inhibitor (C, probably an inversion), together with a gene or genetic condition (s ♀) that sterilizes females homozygous for it, and they also contain bar eye (B). The F_1 females, heterozygous for Cs ♀ B and for the treated wild-type chromosome, were then crossed back to Cs ♀ B males. In the F_2 cultures, the presence or absence of non-bar males was looked for. In this experiment I found, among 394 cultures from females with injured eyes, one lethal, and among 479 parallel control cultures also one lethal. I think our previous work has made it safe to conclude that if lethals have not been produced in an experiment, visibles have not been produced either, the lethal test being the more sensitive one for studies on the general mutation rate.

Up to the present time, therefore, no definite conditions either of an external or of an internal character, except probably temperature, have been found, which influence the general mutation rate in the absence of artificial irradiation. (In speaking of the general mutation rate we are omitting from consideration the eversporting genes, which may represent cases of a special kind.) There are, however, other directions in which we may seek for light on the problem of the relation between X-ray mutations and natural mutations.

IV. Natural Radiation *versus* Other Conditions as the Cause of the Mutations in Nature

As I pointed out in 1927, the manner in which mutation frequency varies with change in dosage is especially im-

portant in its bearing on this question, as well as on the related question of whether or not the effect of X-rays is a direct local effect. At that time, I had not yet been able to accumulate sufficient data to determine this function with any definiteness. Since then Hanson, using radium, and Oliver in our laboratory using X-rays, have both found that the frequency of the mutations produced[9] is exactly proportional to the energy of the dosage absorbed (as indicated by the amount of induced ionization). There is, then, no trace of a critical or threshold dosage beneath which the treatment is too dilute to work. We should expect this result if the effect is a direct effect, locally produced by the electron hits, for no matter how weak a dose is, the released electrons can hold a quantum of energy, and they exert just as strong a local influence as if the total dosage were stronger.

Hanson and Heys's results reported at the present (1929) meeting of the Genetics Sections, showing the independence between the mutation frequency and the wave-length of the X-rays used (the dependence being merely upon the total energy absorbed), are also of great importance in this connection. These, like Hanson's results with radium, prove that any released electron has enough energy to turn the trick, and that we probably can not control the type of mutation, to any great extent at any rate, by controlling the wave-length of the radiation. For if, in response to a shorter wave-length yielding the same total ionization, more mutations of certain kinds were produced than in response to a longer wave-length,

[9] In Oliver's experiments it is especially evident that the proportionality rule holds strictly for the mutations *produced—i.e.*, the remainders obtained by subtracting the frequency in the controls from the frequencies in the treated series are proportional to the dosage, even though the control frequencies vary significantly from one another in different experiments, through unknown causes. The fact that the control frequency comes into the total frequency in this additive way, instead of as a factor by which the dosage factors are multiplied, gives further evidence (like that from temperature previously noted) that the applied radiation does not act like a catalyst, by simply accelerating another, spontaneous process.

and fewer mutations of other kinds, we should scarcely expect the changes in numbers of the different kinds always to counterbalance one another exactly, so as to leave the total numbers of mutations the same.

Now if we conclude that our results are brought about directly by the local electron hits, and that indirect effects are negligible or absent (as most of our present evidence combines to indicate), the corollary follows that the radiation present in nature must produce some at least of the natural mutations. In fact, even if we do not commit ourselves regarding the directness of action of the rays, the direct proportionality between dosage and mutation rate drives us empirically towards this conclusion anyway. This in turn seems at first sight to fit in with the recent finding of Babcock and Collins that flies exposed in a tunnel where radiation was something like twice as strong as in the laboratory showed an increase in number of mutations over those in the laboratory which was two and one half times its own probable error. However, the chance of obtaining such a result if the rates were really equal would have been one in ten,[10] so this result would not as yet be demonstrative even if it were certain that all other factors had been kept constant. This case is different from the apparently parallel findings of Hanson and his colleagues, as I shall try to show presently.

Dr. L. M. Mott-Smith, of the physics department of the Rice Institute, working in collaboration with me, has this fall made calculations of the amount of ionization to which *Drosophila* are subjected when given the doses of X-rays which we customarily use, as compared with the amount to which they are subjected from the ordinary sources of natural high-frequency radiation—of terrestrial and "cosmic" origin combined. Without detailing the figures here, I may say that it turns out that,

[10] We believe the statistical treatment whereby greater odds were arrived at is not applicable to a case of this kind.

in order to account for all the spontaneous mutations on the basis of natural radiation penetrating the flies from their environment, we should have to have terrestrial or cosmic radiation of the order of about 1,000 times as strong as it is ordinarily found to be! In other words, the ordinary natural radiation will account for only about one one-thousandth of the natural mutations that occur in flies, if the frequency of induced mutations is at these low levels of radiation still proportional to the induced ionization, as we have every reason to suppose it to be. We must, therefore, conclude that practically all the mutations that occur in untreated individuals of *Drosophila* are produced by some other cause or causes than the natural radiation present in the general environment.

This being the case, we should not expect any mere doubling, tripling or even a multiplying several tens of times of the weak natural radiation in the general environment to effect any appreciable change in the mutation rate (unless the intake of the organisms also contained more of the radioactive material—a contingency discussed below). In the light of this consideration, then, Babcock and Collins's recent results previously referred to appear rather surprising, and would have to be interpreted as due to some other factor or factors than the relativity slight difference in amount of radiation entering the organisms from their general environment. In Hanson's recent experiment, on the other hand, this consideration probably does not apply, for, in this case, the natural radiation to which the more heavily exposed lot of flies was subjected must have been very many times that in the controls, since the more heavily exposed lot had been placed in a mine of radioactive ore—carnotite. The radioactivity of this carnotite might well have been strong enough to produce an effect upon the mutation rate which could be detected over and above the effect due to the other, unknown, natural cause or causes just demonstrated, which ordi-

narily operate to produce the natural mutations. In view of this other influence (or influences), my work also is affected, for the experiment which I had been making preparations to carry to completion this spring, of excluding most of the natural radiation by shielding flies in a lead box placed in a deep cave, necessarily loses its effectiveness and would yield a negative result.

In spite of these deductions, we are not at this point driven to the conclusion that most of the natural mutations are necessarily due to some cause or causes other than radiation, for we must remember that protoplasm itself has some intrinsic radioactivity. Thus, it certainly contains some potassium, which is very faintly radioactive. J. B. S. Haldane has called our attention to the possible effect of this potassium in producing a "residual" mutation rate. Probably there are also very small amounts of other, far more radioactive substances. Although it would, offhand, seem absurd to suppose that any of these materials would increase the ionization by the necessary thousandfold, nevertheless the amount of effect which they might exert, in augmenting the electronic discharges in the cell and so causing mutations, has not hitherto been estimated.

Calculations of Mott-Smith, based upon previously published data on the radioactivity of potassium, show that ½ per cent. of this substance in the organism (approximately the maximum amount that would occur) would be sufficient to produce only about one thousandth of the mutations that occur in our untreated material—*i.e.*, only about the same amount as the weak natural radiation from outside sources must also be producing. Potassium, then, can not be the cause of most of the natural mutations in flies. The same applies to rubidium. Uranium, too, may be laid aside, because although it is a thousand times more radioactive than potassium, it would itself have to exist in a concentration of ½ per cent. in the organism to account for the spontaneous

mutations; this would be an impossible concentration for uranium. Thorium is far rarer and subject to the same limitations of this kind as is uranium. Practically, we should have left only highly radioactive substances like radium as possible sources of radiation competent to give the observed natural mutation rate. Only about one four-millionth of 1 per cent. of radium would be needed in the organism, to give the effect noted. Does any such concentration of a highly radioactive substance occur?

While our consideration of the above matters was in progress, my attention was called to a paper that had appeared in 1929 by an eminent Russian geochemist, Vernadsky. It was quoted and featured in a recent paper by Timofeëf-Ressovsky.[11] Vernadsky is studying the content of radioactive material in various organisms, and he reports that without exception their content of radium is many fold that of their surrounding environment, so that they may be called veritable "condensers" or collectors of this substance. It is evident that such a property, the tendency to store radium, might well explain the difficulty raised by the inadequacy of outside

[11] After our calculation of the thousandfold inadequacy of the natural radiation in the environment had been made and we were beginning our study of the amount and the possible effectiveness of radioactive substances contained within the organisms, the paper by Timofeëf-Ressovsky, with its reference to the paper by Vernadsky (which was unavailable to us), came to hand. We immediately wrote to Dr. Timofeëf-Ressovsky for further details, and he has kindly prepared for us an English translation of Vernadsky's paper. This translation has just arrived (February 3, 1930)—since the above was written. It appears that in the organisms studied by Vernadsky—land and aquatic plants, and plankton animals—although radium was much more concentrated than in the surroundings, the highest concentration mentioned was only about one twenty-five billionth of 1 per cent. (3.9×10^{-11} per cent.). Other concentrations, while different, were of the same order of magnitude—obviously insufficient. In his letter to us, accompanying the translation, Dr. Timofeëf-Ressovsky states that he is making an investigation similar to ours. The estimate which he had already arrived at of the inadequacy of the natural radiation from outside sources agrees substantially with that which we had reached (page 238).

sources of radiation, and open up in addition a train of curious and important consequences. It might explain not only the relatively (to the natural radiation) far too high natural mutation frequency, but also the apparent epidemics or runs of mutation that have been met with in some batches of control material, and at times, too, as it seems, in particular (non-radiated) individuals, or even particular cells, since under some circumstances cells and organisms would have access to more radium than under others. Sometimes, too, they might be better able to store it, and this would give rise to an apparent effect on the mutation rate occurring in the absence of artificial irradiation, of the conditions responsible for the increased storage power (*e.g.*, temperature?) provided the organisms passed through a sufficient portion of their life cycle under these conditions.

If I may for the moment (without committing myself as to its truth) speculate a little further on this intriguing possibility, I would point out that, if radiation were the one and only cause of mutations, the property in question, radium-storage, would itself become explained and might in fact be regarded as an inevitable consequence of past organic evolution. For in that case, the natural mutability of organisms due to external radiation would probably be very low—as we have calculated above, there would be, in *Drosophila,* not more than one one-thousandth of the spontaneous mutability now observed. Thus this natural mutation frequency would doubtless be so low as to constitute the limiting factor in determining the rate of evolution (*cf.* the recent conclusions of Wright). Variations in the direction of greater radium storage, resulting in a higher mutation rate, would therefore be advantageous, because of their allowing faster evolution, and they would tend in the long run to be preserved and further developed, up to an optimum limit, just as sexual reproduction itself has been preserved and developed on account of its similar value in making faster

evolution possible (in this case, through the recombining of mutations). However, if there were other, readier means than radiation of producing mutations or altering the genic mutability, the property of radium storage would not be expected ordinarily to become strongly developed (certainly not to a degree sufficient to explain the natural mutation-frequency now occurring).

In order to obtain further evidence regarding these matters, Dr. Mott-Smith and I are now conducting an investigation of the content of radioactive substances in flies. Preliminary determinations[12] which he has recently made indicate that the flies do not contain nearly enough radioactive material to explain their natural mutation rate, unless, perhaps, we make the rather implausible assumption that this material is very strongly concentrated in the region of their maturing sex cells. As matters stand at present, therefore, we should be prepared to discount the importance for evolution of the radium storage that occurs, and to accept as more probable the alternative that the great majority of natural mutations have as their primary cause some other process or processes than the absorption of radiation. Thus we seem to be obtaining a negative answer to the query which I raised in 1927 as to whether natural radiation fashions the building-blocks of evolution.

One branch of mutation study would then become involved in the investigation of other ways of producing mutations. Since, however, the mutations produced by the convenient and potent method of radiation are obviously of just the same kind as the others, this latter method will continue to furnish genetics with a most powerful means of studying the mechanism of mutation in general.

As I have pointed out elsewhere, this similarity between the radiation mutations, which must certainly be accidental, and the natural mutations provides cogent

[12] These tests were made, and this paragraph and the next have been added, since the original paper was given.

evidence concerning the accidental nature of the natural mutations also, and hence concerning the method of evolution, regardless of whether or not most of the natural mutations are actually produced by radiation. Hence, too, the radiation method might be used practically to produce "constructive" or advantageous mutations, in material in which such mutations are possible at all. And it can be used by the biologist to furnish genetic alterations of almost all possible kinds that he needs or desires in his work, and then again to provide genetic tools for the study of these alterations themselves. I have devoted my attention in much of the foregoing section to but one phase of the work on radiation and genetics—the relation between natural mutations and our radiation mutations. Important as this is, it is nevertheless only one among many other pressing problems in this new realm of radiation genetics, as I should now like to indicate.

V. Possibilities of Radiation Genetics

Among these other kinds of genetic work which radiation is now making more feasible is that relating to the action of the genes in development. Patterson's recent work on the production of mutant somatic areas by treatment of the developing embryo or larva has admirably illustrated some of the possibilities here. But, on the whole, the surface of this mine can as yet have been only scratched.

Another field that we are only at the edge of is that of gene study: for example, determining what the genes in given loci are capable of if we cause them to mutate again and again, both in parallel (*i.e.*, when the same allelomorph mutates on different occasions to various other allelomorphs) and in series (when a given gene undergoes successive changes from one allelomorph to another); counting and maybe measuring the genes; studying their stabilities; their dominance relationships; their effects in different relative quantities (defi-

ciency and excess); their influence upon the synaptic and other properties of the chromosome or chromosome-region containing them; their possible position-effects if they are shifted in location.

The latter point reminds us of still another broad field of genetics capable of attack through radiation—that of changes in chromosome morphology—displacements of chromatin segments of varied kinds, which in both *Drosophila* and *Datura* are producible as readily as are gene mutations. We have already been able to put the theory of linear linkage on a firmer basis by means of the translocations, duplications and deletions, using them in place of crossovers to obtain verification and correction of our earlier maps of the chromosomes. But it is more interesting to enter new channels. One way in which we are now using them is to trace down the locus of the sex differentiator in the X chromosome. Another is to study the synaptic properties of chromosomes through inversions and translocations: *e.g.*, I have found recently that in *Drosophila* when a large segment of a chromosome is translocated onto a non-homologous chromosome, the two pairs of chromosomes involved no longer undergo a random assortment. Like parts tend to synapse, even when attached to unlike parts that bear the spindle fiber, and the direction of migration at the segregation division is influenced thereby. As a result, there is what may be called a "partial chromosome linkage" in *Drosophila*, much like the more complete linkage which Darlington has assumed to have been caused by translocations in *Oenothera*, and which Blakeslee and his coworkers have found in *Datura*. It may be objected that in the latter cases not simple translocation, but mutual translocation, segmental interchange, is dealt with, but I find that this phenomenon too is produced in *Drosophila* by X-rays, though the relative frequencies of the two kinds of translocations—simple and mutual—are as yet undetermined.

Fortunately for our interest in our subject, it is not possible neatly to map out or codify all the possibilities

of the work, for new and unexplained phenomena have not ceased turning up. To leave you with this thought by way of conclusion, let me briefly refer to the ever-sporting phenomena, of which we have obtained some score of different cases in our laboratory. On a previous occasion I have mentioned a mottled eye produced after X-raying, that acted as an allelomorph of white and was accompanied by a Notch wing effect dependent on another, nearby locus. The mottling is almost certainly due to a genetic variability in the somatic tissue, because there is also a germinal instability, evidenced by many of these chromosomes failing to breed true, and giving darker and lighter genetic types in which there are correlated changes in the notching of the wing. I should mention also that the somatic mottling includes a variegation in the coloration of the testes as well as in that of the eye. The "genomere" hypothesis of gene structure does not apply to explain these results because the darker and lighter races produced are still somewhat mottled themselves—there is no sorting out to the uniform condition. Moreover, an intragenic sorting out could scarcely be conceived as affecting both the mottled and the Notch locus at once. Now this mottled "factor" lies in a chromosome segment that has been displaced—translocated. Since this case was found, various other mottled allelomorphs of white have been produced in my experiments. Though they are not notched winged, they are mottled eyed in similar fashion. The important point is that in every one of these cases there has been a gross displacement—a breakage and reattachment—of the chromosome segment bearing the locus in question. In some cases, the displacement is a translocation to a non-homologous chromosome, in other cases an inversion, in another case a deletion has occurred—the type of displacement, in this sense, does not matter, but there must be a displacement.

To explain a case of this sort, we can not assume that the recently reattached segment is especially likely to

break off again and then be lost, the loss resulting in the lighter patches, for in a male the loss of a part of the X chromosome would, we have every reason to infer, result in the death of the cell. The displaced segment must therefore undergo some other kind of "gyration": either reduplication or diminution or relocation, or some other antics of a previously unknown sort. Our findings here are not confined to the locus mentioned. Other variegated eye conditions have been found, involving autosomal loci, and also a variegated bristle condition or two, and these also in every case investigated involve displacements of chromosome sections. Some are accompanied by a genetic eversportingness similar to that already described for the first mottled. Moreover, they include the only dominant eye-color changes known in *Drosophila melanogaster*. I do not call these cases of eversporting genes; they are eversporting displacements. Whether any previously described cases of eversporting genes really belong in this category can not yet be decided. Here, then, an alluring and unsolved problem has appeared that does not fit in with the list of possibilities that we might have preconceived in an *a priori* outline for a radiation program.

Radiation and genetics is my title, but I can not do it justice, for the field of radiation genetics is, in a sense, coextensive with that of genetics itself—it affects or induces crossing over, non-disjunction, chromatin displacements, gene-mutation and even, as Patterson has recently found, somatic segregation. And it produces these things in quantities, under determinate conditions. If ever you are *ennuied,* just try rubbing the Aladdin's lamp of the X-ray tube or the radium needle, and pretty soon you may be flooded with a "superfluity of riches," in the midst of which the chief question becomes, what not to ignore. But be forewarned that, like a drug, this may completely spoil you for any other mode of life.

The author wishes to acknowledge with appreciation the assistance of a grant from the committee on the

effects of radiation on living organisms of the National Research Council in the carrying out of more recent work reported in this paper.

BIBLIOGRAPHY OF PAPERS SUBSEQUENT TO SEPTEMBER, 1928

1. Altenburg, E.
1928. "The Limit of Radiation Frequency Effective in Producing Mutations," Am. Nat., 62: 540–545.
2. Anderson, E. G.
1929. "Studies on a Case of High Non-disjunction in *Drosophila melanogaster*," *Zeit. ind. Abst. u. Vererb.*, 51: 397–441.
3. Avery, A. G., and Blakeslee, A. F.
1928. "Radium Experiments with *Datura*. IV. Visible Gene Mutations in Radium-treated and Control Material" (Abstr.), *Anat. Rec.*, 41: 99.
4. Babcock, E. B., and Collins, J. L.
1929. "Does Natural Ionizing Radiation Control Rate of Mutation?" *Proc. Nat. Acad. Sc.*, 15: 623–628.
5. Babcock, E. B., and Collins, J. L.
1929. "Natural Ionizing Radiation and the Rate of Mutation," *Nature*, 124: 227–228.
6 Barth, L. G.
1928. "The Effect of X-rays on the Spermatozoa of *Drosophila*" (Abstr.), *Anat. Rec.*, 41: 117.
7. Bergner, A. D., Satina, S., and Blakeslee, A. F.
1928. "Radium Experiments with *Datura*. III. Chromosomal Abnormalities in F_1's from Radium-treated Parents" (Abstr.), *Anat. Rec.*, 41: 99.
8. Blakeslee, A. F., Bergner, A. D., Satina, S., Avery, A. G., Cartledge, J. L., and Potter, J. S.
1929. "Analysis of *Datura stromonium* Plants Grown from Seeds Exposed to X-rays" (Abstr.), *Anat. Rec.*, 44: 281.
9. Buchholz, J. T., and Blakeslee, A. F.
1928. "Radium Experiments with *Datura*. I. The Identification and Transmission of Lethals of Pollen-tube Growth in F_1's from Radium-treated Parents" (Abstr.), *Anat. Rec.*, 41: 98.
10. Buchholz, J. T., and Blakeslee, A. F.
1928. "Radium Experiments with *Datura*. V. Effects of Radium on Pollen-tube Growth Rate, and the Seed Output Following Treatment" (Abstr.), *Anat. Rec.*, 41: 101.
11. Cartledge, J. L., and Blakeslee, A. F.
1928. "Radium Experiments with *Datura*. II. Pollen Abortion in F_1's from Radium-treated Parents" (Abstr.), *Anat. Rec.*, 41: 98.
12. Casteel, D. B.
1929. "Histology of the Eyes of X-rayed *Drosophila*," *Jour. Exp. Zool.*, 53: 373–385.

12a. Clark, G. L., and Boruff, C. S.
1929. "The Effect of X-rays on Bacteria," *Science*, 70: 74–75.
13. Davenport, C. B.
1929. "Annual Report of the Director of the Department of Genetics," Carn. Inst. Wash. Yr. Bk. 28: 33–66.
13a. Dobrovolskaia-Zavadskaia, N.
1929. "The Problem of Species in View of the Origin of Some New Forms in Mice," *Biol. Rev.*, 4: 327–351.
14. Dobzhansky, Th.
1929. "A Homozygous Translocation in *Drosophila melanogaster*," *Proc. Nat. Acad. Sci.*, 15: 633–638.
15. Dobzhansky, Th.
1929. "Genetical and Cytological Proof of Translocations Involving the Third and Fourth Chromosomes of *Drosophila melanogaster*," *Biol. Zentr.*, 49: 408–419.
16. Dubinin, N. P.
1929. "Allelomorphentreppen bei *Drosophila melanogaster*," *Biol. Zentr.*, 49: 328–339.
17. Fischer, E.
1929. "Strahlenbehandlung und Nachkommenschaft," *D. Med. Woch.*, no. 3, pp. 1–6.
18. Goodspeed, T. H.
1929. "The Effects of X-rays and Radium on Species of the Genus *Nicotiana*," *Jour. Hered.*, 20: 243–259.
19. Goodspeed, T. H., and Olson, A. R.
1928. "Progenies from X-rayed Sex-cells of Tobacco," *Science*, 67: 46.
19a. Gowen, J. W.
1929. "On the Nature of Gene Action," *Science*, 70: 358–360.
20. Grüneberg, H.
1929. "Ein Beitrag zur Kenntnis der Röntgenmutationen des X-Chromosoms von *Drosophila melanogaster*," *Biol. Zentr.*, 49: 680–694.
21. Haldane, J. B. S.
1929. "The Species Problem in the Light of Genetics," *Nature*, 124: 514–516.
22. Hanson, F. B.
1928. "An Analysis of the Effects of the Different Rays of Radium in Producing Lethal Mutations in *Drosophila*" (Abstr.), *Anat. Rec.*, 41: 99.
23. Hanson, F. B., and Heys, F.
1929. "An Analysis of the Effects of the Different Rays of Radium in Producing Lethal Mutations in *Drosophila*," AM. NAT., 63: 201–213.
24. Hanson, F. B., and Heys, F.
1929. "Duration of the Effects of X-rays on Male Germ-cells in *Drosophila melanogaster*," AM. NAT., 63: 511–516.

25. Hanson, F. B., and Heys, F.
1929. "The Effect of Natural (Earth) Radiation in Producing Lethal Mutations in *Drosophila melanogaster*" (Abstr.), *Anat. Rec.*, 44: 293.
26. Hanson, F. B., and Heys, F.
1930. "A Possible Relation between Natural (Earth) Radiation and Gene Mutations," *Science*, 71: 43–44.
27. Hanson, F. B., and Stanton, E.
1929. "The Effect of Increasing X-ray Voltages on the Production of Lethal Mutations in *Drosophila melanogaster*" (Abstr.), *Anat. Rec.*, 44: 293.
28. Hanson, F. B., and Winkleman, E.
1929. "Visible Mutations Following Radium Irradiation in *Drosophila melanogaster*," *Jour. Hered.*, 20: 277–286.
29. Harris, B. B.
1929. "The Effects of Aging of X-rayed Males upon Mutation Frequency in *Drosophila*," *Jour. Hered.*, 20: 299–302.
30. Joly, J., and H. H. Dixon
1929. "Cosmic Radiations and Evolution," *Nature*, 123: 981.
31. Mavor, J. W.
1929. "X-rays and Mutations," *Science*, 69: 500–501.
31a. Mavor, J. W.
1929. "The Effect on Crossing Over and Non-disjunction of X-raying the Anterior and Posterior Halves of *Drosophila* Pupae," *Genetics*, 14: 129–159.
32. Morgan, T. H., Sturtevant, A. H., and Bridges, C. B.
1928. "The Constitution of the Germinal Material in Relation to Heredity," Carn. Inst. Wash. Yr. Bk. 27: 330–335.
33. Muller, H. J.
1928. "The Production of Mutations by X-rays," *Proc. Nat. Acad. Sc.*, 14: 714–726. (See this paper for bibliography of papers on the subject previous to those listed in the present bibliography.)
34. Muller, H. J.
1929. "The First Cytological Demonstration of a Translocation in *Drosophila*," AM. NAT., 63: 481–486.
35. Muller, H. J.
1929. "The Method of Evolution," *Sci. Mo.*, 29: 481–505.
36. Muller, H. J.
1930. "Types of Visible Variations Produced by X-rays in *Drosophila*," *Jour. Gen.* (in press).
37. Muller, H. J.
1930. "Oenothera-like Linkage of Chromosomes in *Drosophila*," *Jour. Gen.* (in press).
38. Muller, H. J., and Altenburg, E.
1928. "Chromosome Translocations Produced by X-rays in *Drosophila*" (Abstr.), *Anat. Rec.*, 41: 100.
39. Muller, H. J., and Altenburg, E.
1930. "The Frequency of Translocations Produced by X-rays in *Drosophila*," *Genetics* (in press).

39a. Muller, H. J., and Mott-Smith, L. M.
1930. "Evidence that Natural Radioactivity is Inadequate to Explain the Frequency of (Natural) Mutations" (in press).

40. Muller, H. J., and Painter, T. S.
1929. "The Cytological Expression of Changes in Gene Alignment Produced by X-rays in *Drosophila*," AM. NAT., 63: 194–200.

40a. Nadson, G. A., and Philippov, G. S.
1928. "De la formation de nouvelles races stables chez les champignons inférieurs sous l'influence des rayons X," *C. R. de l'Acad. des Sci.*, 186: 1566–1568.

41. Oliver, C. P.
1930. "The Effect of Varying the Duration of X-ray Treatment upon the Frequency of Mutation," *Science*, 71: 44–46.

42. Olson, A. R., and Lewis, G. N.
1928. "Natural Radioactivity and the Origin of Species," *Nature*, 121: 673–674.

43. Painter, T. S., and Muller, H. J.
1929. "The Parallel Cytology and Genetics of Induced Translocations and Deletions in *Drosophila*," *Jour. Hered.*, 20: 287–298.

44. Patterson, J. T.
1929. "X-rays and Somatic Mutations," *Jour. Hered.*, 20: 261–268.

45. Patterson, J. T.
1929. "The Production of Mutations in Somatic Cells of *Drosophila melanogaster* by Means of X-rays," *Jour. Exp. Zool.*, 53: 329–372.

46. Patterson, J. T.
1930. "Somatic Segregation Produced by X-rays in *Drosophila melanogaster*," *Proc. Nat. Acad. Sci.* (in press).

47. Patterson, J. T.
1930. "Proof that the Entire Chromosome is Not Eliminated in the Production of Somatic Variations by X-rays in *Drosophila*," *Genetics*, 15: 141–149.

48. Serebrovsky, A. S.
1929. "A General Scheme for the Origin of Mutations," AM. NAT., 63: 474–478.

49. Serebrovsky, A. S., and Dubinin, N. P.
1930. "X-ray Experiments with *Drosophila*," *Jour. Hered.* (in press).

50. Serebrovsky, A. S., Dubinin, N. P., Agol, I. I., Sliepkoff, W. N., and Altshuler, W. E.
1928. "Poluchenie mutatsii roentgenovskimi luchami u *Drosophila melanogaster*," *Jour. Exp. Biol.* (Russ.), A 4: 161–180.

51. Stadler, L. J.
1928. "The Rate of Induced Mutation in Relation to Dormancy, Temperature and Dosage" (Abstr.), *Anat. Rec.*, 41: 97.

52. Svenson, H. K.
1929. "On the Biological Effects of X-rays," *Science*, 69: 361.
53. Timofeëf-Ressovsky, H. A.
1930. "The Effect of X-rays upon the Mutability of *Drosophila funebris*," *Jour. Hered.* (in press).
54. Timofeëf-Ressovsky, N. W.
1929. "The Effect of X-rays in Producing Somatic Genovariations of a Definite Locus in Different Directions in *Drosophila melanogaster*," AM. NAT., 63: 119–124.
55. Timofeëf-Ressovsky, N. W.
1929. "Rückgenovariationen und die Genovariabilität in verschiedenen Richtungen. I. Somatische Genovariationen der Gene W, we und w bei *Drosophila melanogaster* unter dem Einfluss der Röntgenbestrahlung," *Roux' Arch. f. Entwmech.*, 115: 620–635.
56. Timofeëf-Ressovsky, N. W.
1929. "Somatic Genovariations of a Definite Gene in Different Directions under the Influence of Roentgen Radiation" (Russ.), Trudy Vsesoj. Syezda po Genetike i Selectsii.
57. Timofeëf-Ressovsky, N. W.
1929. "Der Stand der Erzeugung von Genovariationen durch Röntgenbestrahlung," *Jour. f. Psych. u. Neurol.*, 39: 432–437.
58. Timofeëf-Ressovsky, N. W.
1930. "The Production of Reverse Genovariations in *Drosophila melanogaster* by X-ray Treatment" (in press).
59. Torvik, M. M.
1929. "Are Biparental Males of *Hadrobracon* Diploid for All Chromosomes?" (Abstr.), *Anat. Rec.*, 44: 286.
60. Vernadsky, V. I.
1929. "On the Concentration of Radium in Living Organisms" (Russ.), C. R. de l'Acad. d. Sci. d. U. S. S. R., A 2.
61. Weinstein, A.
1928. "Genetic Effects of X-rays and Other Environmental Agents" (Abstr.), *Anat. Rec.*, 41: 100.
62. Whiting, P. W.
1928. "Heredity of Miniature (Body), an X-ray Mutation in *Hadrobracon*" (Abstr.), *Anat. Rec.*, 41: 106.
63. Whiting, P. W., and Whiting, A. R.
1929. "The Mutant Types, X-ray and 'Natural,' of *Hadrobracon*" (Abstr.), *Anat. Rec.*, 44: 285.
64. Wright, S.
1929. "Evolution in a Mendelian Population" (Abstr.), *Anat. Rec.*, 44: 287.

THE CHLOROPHYLL FACTOR IN PHOTOSYNTHESIS[1]

DR. ROBERT EMERSON
HARVARD UNIVERSITY

OF all the natural processes dependent on the radiation received from the sun, photosynthesis is probably the most accessible and easily studied. This process is the reduction of carbon dioxide by green plants to form carbohydrates. The carbohydrates thus produced contain much more energy than the carbon dioxide and water from which they were made. This increase in energy is derived from the sun's radiation which falls on green plants, and without which no synthesis of carbohydrates takes place. Photosynthesis is a process by which the sun's radiant energy is stored in chemical compounds produced by the green plant. Were it not for this storage process, a large portion of the energy of sunlight falling on the earth would be dissipated at once, as heat for the most part. As it is, however, this radiant energy is stored in chemical compounds and released gradually, supporting animal life and making possible the undertakings of man.

Playing, as it does, this important part in nature, photosynthesis has attracted the interest of scientists ever since it was correctly interpreted by Priestley, Lavoisier and a few others. Probably more precise knowledge has been accumulated about photosynthesis than about any other natural process depending on radiation.

The usual source of radiation for green plants is the sun. The energy of this radiation varies greatly with the frequency or wave-length. It seems reasonable that the eye should be adjusted to sense those frequencies of radiation in which sunlight is richest, and we call this particular range of frequencies the visible spectrum. Photosynthesis does not make use of all frequencies of the

[1] Read before the American Society of Naturalists, Des Moines, Iowa, January 1, 1930, as a part of the symposium on "Radiation and Life."

sun's radiation but is adjusted to those frequencies richest in energy, included in the visible spectrum. It goes on throughout the visible but not in ultra-violet or infrared radiation.

The raw materials of photosynthesis, carbon dioxide and water, are familiar to us as colorless substances. This means that they absorb no visible light. It is a fundamental law of photochemistry that light which is not absorbed can not bring about any reaction. In order to have the rich energy of visible light available for a reaction between two colorless substances, carbon dioxide and water, there must be some colored substance to absorb the light and transfer the energy to the two reactants. This substance is chlorophyll, the principal coloring matter of green plants.

Recently it has been claimed that photosynthesis with visible light has been achieved through the agency of certain colored inorganic salts. Although such reactions may be possible, I do not believe that they are as yet satisfactorily demonstrable, so for our purposes we may consider chlorophyll as the only known photosensitizer causing photosynthesis in visible light. This is independent of reactions between carbon dioxide and water in ultra-violet light, which probably proceed in a way completely different from photosynthesis in visible light.

We have in photosynthesis a reaction caused by radiation, in which a number of the partaking substances are known chemical compounds. We have a reaction between carbon dioxide and water, brought about through the activity of chlorophyll, a colored substance of definite chemical composition. Recent work has established that the first intermediate product of the reaction is formaldehyde, and we know that the final product is carbohydrate, usually some sort of sugar. From the investigations of Warburg we know the amount of light-energy necessary for the reduction of a given amount of carbon dioxide in different parts of the spectrum. There is no other biological process or reaction caused by radiation about which we have so much definite information or which is

so accessible to study. Nevertheless, we are still in comparative ignorance about the mechanism and kinetics of the reaction.

I should like to direct attention to chlorophyll, the absorber of the radiation essential for the process. We know that plants or parts of plants which contain no chlorophyll can not carry on photosynthesis, but aside from this we have no evidence that chlorophyll is really the only absorber of radiation. It is always accompanied by several other pigments.

Willstätter pointed out about ten years ago that the only way we can hope to elucidate the function of chlorophyll in photosynthesis is through a study of the process at different concentrations of chlorophyll per unit area of leaf or per unit volume of cell substance. If we could establish a precise correspondence between the amount of chlorophyll and the rate of photosynthesis, this would give us additional evidence that chlorophyll is actually the photosensitizer.

Willstätter's own studies in this direction, although extensive and very interesting, fail to show any regular connection between the amount of chlorophyll present per assimilating unit and the rate of photosynthesis. Surely we should expect to find some relationship between these two if chlorophyll plays any part at all. I believe Willstätter failed to find a relationship because he used leaves different in many respects besides chlorophyll content. In order to obtain material showing a sufficient range of chlorophyll concentrations, he used young and old leaves, leaves from shade and sunlight plants, yellow varieties, etc. It is hardly strange that he found that the photosynthetic activity of these leaves bore no relation to the amounts of chlorophyll they contained.

Willstätter made one attempt to grow leaves of equal ages of the same species with different amounts of chlorophyll. He raised seedlings on nutrient media containing different amounts of iron salts. Such seedlings show different degrees of chlorosis, but unfortunately are not normal in other respects. The plants grown on the media

poorest in iron have a limited growth and produce small, spindly leaves. This is natural, since they need carbohydrates to provide energy for growth, and as soon as they have used up what is stored in the seed and have become too chlorotic to produce more they are starved.

If we choose an organism to which we can feed carbohydrates when it is more or less deprived of the ability to synthesize them, we can overcome this abnormality and maintain at least comparatively normal growth in chlorotic cultures. This has been done successfully with pure cultures of a unicellular green alga, *Chlorella,* using glucose as a carbohydrate. Such cultures may be grown to show any desired degree of greenness, depending on the amount of iron added. Cultures poorest in iron show no chlorophyll at all and have only the bright yellow color of the pigments usually accompanying chlorophyll. Except for color the yellow cells are apparently normal in all important respects. They are of normal size and show normal respiration as compared to green cells grown in glucose. Their growth is somewhat slower than that of green cultures with adequate amounts of iron, but it does not stop entirely, like the growth of the chlorotic seedlings.

It should be understood that the cells without chlorophyll are not grown on media free from iron. This element is essential for growth and respiration. But far more iron is necessary for normal chlorophyll production than for normal respiration and growth.

We can compare, then, the rates of photosynthesis of sets of cells similar in all essential respects except for the amounts of chlorophyll they contain. The chlorophyll can be extracted and its concentration per unit volume of cells can be determined spectrophotometrically. The rates of photosynthesis when plotted against chlorophyll concentrations show such curves as are drawn in Fig. 1. These curves indicate that the rate of photosynthesis is nearly proportional to the amount of chlorophyll per unit volume of cells. I believe this is the first quantitative evidence that chlorophyll is the photosensitizer for carbon dioxide assimilation.

The rates of photosynthesis for these curves were measured at a light intensity so high that for normally green cells a decrease to one half the intensity caused no appreciable decrease in the rate of photosynthesis. The cells were more than saturated with light. Yet we find that a decrease in chlorophyll content by much less than one half causes a considerable fall in the rate of photosynthesis. If a given decrease in light intensity causes no decrease in rate, we should hardly expect the same decrease in the amount of light-absorber to cause any decrease in rate.

Willstätter's results suggest that a higher intensity of light is necessary to saturate leaves poor in chlorophyll than is required for leaves rich in chlorophyll. This might mean that a higher intensity should be used for the measurements with lower chlorophyll content, in order to show always maximum possible rates of photosynthesis. As a matter of fact, all measurements for Fig. 1 were made at the same light intensity. Fig. 2 shows that this

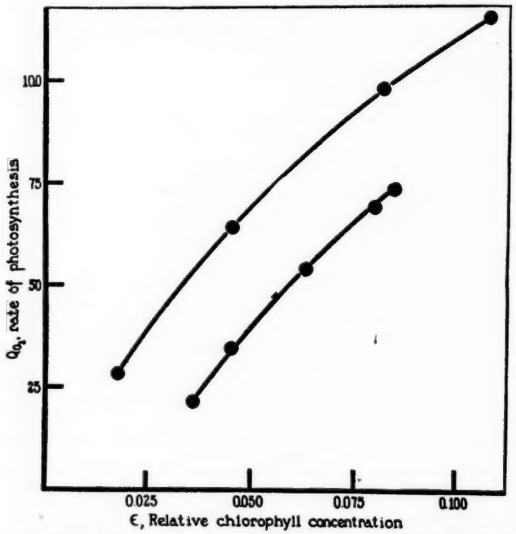

FIG. 1. Curves showing the rate of photosynthesis at different concentrations of chlorophyll per unit volume of cells.

procedure is justified. Cells poor in chlorophyll reach their maximum rate of photosynthesis at nearly the same light intensity as normal cells.

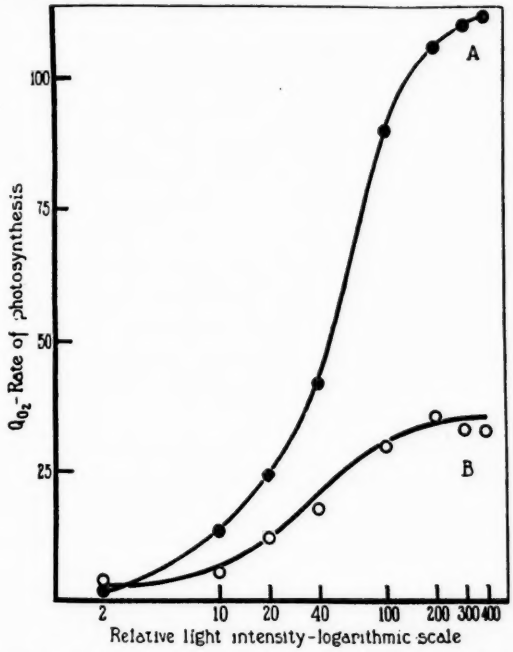

FIG. 2. Curves showing rate of photosynthesis as a function of light intensity, for two different concentrations of chlorophyll per unit volume of cells. The cells used for curve A contained about four times as much chlorophyll per unit volume as those used for curve B.

Photosynthesis thus behaves in different ways when light intensity is cut down and when chlorophyll content is decreased. We might compare the chlorophyll factor and the light factor in another way. At high light intensities photosynthesis shows a sensitivity to temperature which almost vanishes at low light intensities. The following explanation for this phenomenon is generally accepted. At least two reactions are involved in photosynthesis, a photochemical reaction whose rate is independent of temperature and governed by light intensity, and a reaction of chemical or enzymatic character whose rate

is dependent on temperature but independent of the light intensity. Since F. F. Blackman was the first to make many of the observations and measurements on which this explanation is based, the second, or enzymatic, reaction is usually called the Blackman reaction.

It is generally agreed that the photochemical precedes the Blackman reaction and furnishes the latter with substrate. If the light intensity is low, the photochemical reaction will proceed so slowly that the enzyme of the Blackman reaction will not be working at its maximum rate. Hence temperature will not affect the rate of the process as a whole. But if the light intensity is so high that the photochemical reaction is furnishing the Blackman reaction with much more substrate than it can use, then changes in temperature will immediately affect the rate of the process as a whole, which is dependent always on the slowest reaction.

Cutting down the light intensity presumably causes the temperature effect to vanish because the rate of formation of the substrate for the Blackman reaction is cut down. Lowering the chlorophyll concentration should decrease the rate at which the intermediate product is formed, just as does cutting down the light intensity. But Fig. 3 shows that there is practically no loss of sensitivity to temperature changes, even at very low chlorophyll contents. Decreasing the chlorophyll content again shows a different effect from cutting down the light intensity.

Besides being sensitive to temperature changes, the Blackman reaction is excessively sensitive to traces of prussic acid. The photochemical reaction is much less affected by the same concentrations of prussic acid, as shown by the fact that the rate of photosynthesis at low light intensities is hardly affected, while at high intensities the rate is strongly inhibited by minute amounts of this acid. The sensitivity of photosynthesis to prussic acid remains at low chlorophyll concentrations, instead of disappearing, as it does with diminished light intensity.

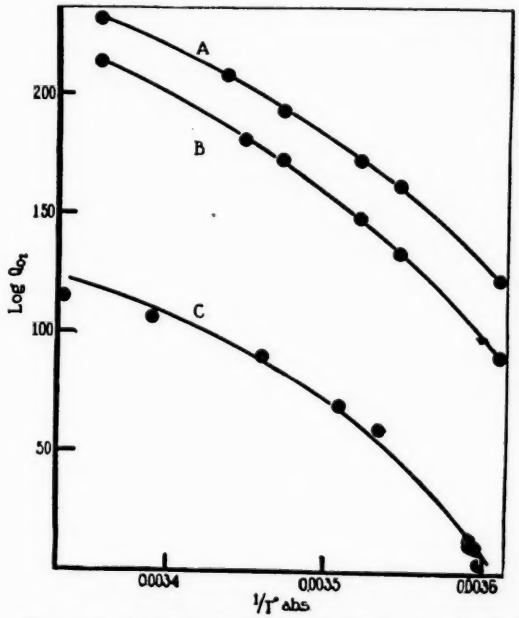

FIG. 3. Curves showing the effect of temperature on rate of photosynthesis at three different concentrations of chlorophyll.

It is unlikely that the primary photochemical reaction is different at high and low chlorophyll concentrations. It may be that the cells poor in chlorophyll are less well equipped with the enzyme of the Blackman reaction. It is impossible to be certain about this point as yet, but we can apply one test of the capacity of cells to carry on the Blackman reaction. Warburg and Uyesugi have shown that this reaction probably consists in the splitting of a peroxide. *Chlorella* cells are equipped with an enzyme which splits hydrogen peroxide readily, and this reaction shows many of the characteristics of the Blackman reaction. *Chlorella* cells poor in chlorophyll or even without chlorophyll show an undiminished capacity to split hydrogen peroxide, so at least we can say there is no evidence for thinking that such cells are less well equipped with the enzyme of the Blackman reaction.

The only other possibility is that chlorophyll itself is concerned in the Blackman reaction, as well as being the

absorber of light for the photochemical reaction. This would fully explain why decreasing the light intensity, which can affect the photochemical reaction only, should give different results from diminishing the chlorophyll content, which I believe affects the Blackman reaction as well as the photochemical reaction. In other words, chlorophyll must play the part of a chemical reactant in photosynthesis, as well as being the photosensitizer which absorbs the radiant energy necessary to the process.

SUMMARY

Photosynthesis is a process taking place in visible light and involving a reaction between two colorless substances, carbon dioxide and water, which gives rise to carbohydrates. The process is made possible in visible light by the presence of chlorophyll, the chief coloring matter of green plants. The function of chlorophyll can be revealed only by a study of the process when different amounts of chlorophyll are present. This condition is made possible by applying appropriate culture methods to the unicellular green alga *Chlorella*. *Chlorella* cells may be produced which show all degrees of greenness but which are normal in other respects. A study of their capacity for photosynthesis at different concentrations of chlorophyll per unit volume of cells, using different light intensities and temperatures, indicates that chlorophyll plays the part of a chemical reactant in photosynthesis as well as being the absorber of energy in the form of visible light, which furnishes the driving force for the process.

LITERATURE CITED

E. C. C. Baly
1927. *Proc. Roy. Soc. A.*, 116: 219.

Robert Emerson
1927. *Jour. Gen. Physiol.*, 10: 469.
1929. *Jour. Gen. Physiol.*, 12: 609, 623.

Otto Warburg
1925. *Biochem. Zeit.*, 166: 386.

Willstätter and Stoll
1918. "Untersuchungen über die Assimilation der Kohlensäure," Berlin.

GENETICAL AND ENVIRONMENTAL FACTORS INFLUENCING THE TYPE OF INTERSEXES IN DROSOPHILA MELANOGASTER

T. DOBZHANSKY
CALIFORNIA INSTITUTE OF TECHNOLOGY

IF a triploid female is crossed to a diploid male, a part of the offspring consists of individuals possessing two X-chromosomes and three sets of autosomes. These individuals are intersexes. According to Bridges (Bridges, 1921, 1922, 1923; Morgan, Bridges and Sturtevant, 1925), the intersexuality is due in this case to the ratio between the number of X-chromosomes and the number of sets of autosomes. This ratio ($2:3=0.67$) is intermediate between that observed in females ($2:2=1.00$) and that observed in males ($1:2=0.50$).

Considered morphologically, intersexes exhibit an extraordinarily high variability in both external and internal structures. The extreme male-type intersexes are very similar to normal males in appearance and possess normal-looking male reproductive organs, although the spermatozoa formed are non-functional. On the other hand, the extreme female-type intersexes are similar to normal females but are rather smaller in size, in most cases have sex-combs, and do not produce functional ova. These two extremes are linked by a long series of intermediate types. The intermediate types of intersexes are, however, not intergrades in the proper sense of the word, but are mixtures of nearly typical male and typical female characteristics in various proportions (Dobzhansky and Bridges, 1928). As far as the reproductive system of the intermediate types of intersexes is concerned, it is in some cases simply reduced to a small vestige and in other cases is composed of some male parts (*e.g.*, penis, genital arch, male-like gonads) together with some female parts (spermathecae, vagina).

The study of the morphological features of the various types of intersexes in connection with the study of the development of the sexual characteristics in normal males and females has shown (Dobzhansky and Bridges, 1928) that the triploid intersexes in Drosophila are in some respects similar to the intersexes in *Lymantria dispar* described by Goldschmidt (Goldschmidt, 1920, 1927). In both cases intersexes may be interpreted as individuals which developed up to a certain moment along the lines characteristic for one sex and thereafter developed along the lines characteristic for the opposite sex. The triploid intersexes of Drosophila develop to a certain moment as males and after that as females. Hence, a Drosophila intersex becomes more female-like if the moment of the reversal of development occurs early, and becomes more male-like if this moment occurs late in the individual history.

It may be asked, however, what is the cause of the diversity of types observed among the intersexes in Drosophila? In other words, what are the factors causing the moment of the reversal in the development to occur earlier or later? At first, the variability observed among the intersexes was attributed by Bridges to the fact that some of them possess three fourth chromosomes while others possess only two fourth chromosomes. This hypothesis was later rejected by Bridges (Dobzhansky and Bridges, 1928), and the variability of intersexes explained by the influence of genetical modifiers. Indeed, most of the intersexes found in cultures of triploids which were bred in the laboratory for many generations possess only two fourth chromosomes, but nevertheless such intersexes exhibit often a great diversity of types. On the other hand, some strains of triploids produce more male-like and other strains more female-like intersexes on the average.

The existence of genetical modifiers influencing the type of intersexes was ascertained by the following experiments. A stock of triploid females which produced

very diverse types of intersexes was obtained through the kindness of Dr. H. Redfield. Several triploid females from this stock were crossed in individual cultures to diploid males from the same stock. In the next generation the culture which produced on the average more male-type intersexes and the culture which produced on the average more female-type intersexes were selected. These two cultures served as progenitors of the two separate strains within one of which selection was carried on toward the production of male-type and in the other of female-type intersexes. In each generation several triploid females were selected from the culture which produced the highest proportion of the desired type of intersexes, and these females were crossed to their brothers. By such a method the selection was carried as far as the twenty-third generation from the beginning of the experiment.

In order to secure numerical data on the progress of selection, the intersexes obtained in each generation were classified into five classes, which may be characterized as follows:

Class 1: Extreme male-type intersexes. External male genitalia are present. Penis and genital arch are symmetrical.

Class 2: External male genitalia or their rudiments are present. Penis and genital arch are asymmetrical, reduced in size or rudimentary.

Class 3: Neither male nor female external genitalia are present.

Class 4: External female genitalia are present at least in rudimentary form. Vaginal plates asymmetrical.

Class 5: External female genitalia are present. Vaginal plates are symmetrical.

It may be seen that the diagnoses of these five classes are based solely on the characters of the external genitalia. However, such a classification of intersexes is by no means "unnatural," since the characters of external genitalia show a high correlation with the characters of the internal parts of the reproductive system as well as with the secondary sexual characters) form and color of the tip of the abdomen, presence or absence of the sex-combs, etc.). Indeed, symmetrical male genitalia (class

1) occur in individuals having the internal sexual organs more or less similar to those of normal males. Asymmetrical or rudimentary external male genitalia (class 2) accompany various degrees of reduction of the internal reproductive system of the male type, including complete disappearance of the male ducts. Absence of the external genitalia of either sex (class 3) corresponds in most cases to the maximal reduction of the internal parts of the reproductive system or to the beginning of the formation of the female ducts (presence of the spermathecae, rudiments of vagina). Finally, presence of the external genitalia of the female type (classes 4 and 5) is correlated with various degrees of the formation of the internal female organs.

TABLE I

SELECTION OF STRAINS PRODUCING MALE-TYPE AND FEMALE-TYPE INTERSEXES

	Intersexes of the initial population											
	1		2		3		4		5		n	
	36.7		43.1		7.8		11.0		1.4		283	
Generation	per cent.		per cent.		per cent.		per cent.		per cent.			
	Female-type line						Male-type line					
	1	2	3	4	5	n	1	2	3	4	5	n
1	13.5	44.6	10.8	27.0	4.1	148	44.8	42.3	7.1	5.2	.5	210
2	8.8	32.4	30.9	24.3	3.7	136	50.6	35.6	7.7	4.6	1.5	261
3	7.0	19.0	21.8	41.5	10.6	142	58.2	27.1	9.4	5.3	—	170
4	10.5	17.0	28.0	39.0	5.5	199	64.0	21.1	8.8	6.1	—	147
5	1.5	8.2	22.9	46.4	20.9	196	83.9	13.3	2.8	—	—	180
6	3.0	15.7	40.4	35.5	5.4	166	74.5	16.3	7.7	1.5	—	208
7	1.3	11.8	32.0	44.4	10.5	153	86.6	11.9	1.5	—	—	201
8	1.4	4.1	58.1	31.1	5.3	74	69.5	21.4	7.1	2.0	—	210
9	2.1	3.2	5.3	57.9	31.6	95	89.5	10.5	—	—	—	219
10	3.6	5.5	15.7	67.3	7.9	165	92.2	7.3	.5	—	—	193
11	3.3	10.5	27.4	49.7	9.2	153	97.9	2.1	—	—	—	190
12	—	2.1	3.1	67.7	27.1	96	95.1	4.9	—	—	—	101
13	—	3.8	30.8	50.0	15.4	26	96.0	2.7	1.3	—	—	75
14	—	6.4	29.8	53.2	10.6	47	95.2	3.8	1.0	—	—	210
15	—	7.0	54.4	33.3	5.3	57	97.3	2.7	—	—	—	223
16	—	1.3	27.6	61.9	9.2	76	98.9	1.1	—	—	—	188
17	—	1.8	33.9	51.8	12.5	112	99.3	.7	—	—	—	138
18	—	—	11.1	66.7	22.2	54	98.6	1.4	—	—	—	219
19	—	1.5	9.0	64.2	25.3	67	99.1	.9	—	—	—	113
20	—	2.3	13.6	69.3	14.8	88	100.0	—	—	—	—	116
21	—	—	5.3	71.9	22.8	57	100.0	—	—	—	—	89
22	—	—	4.1	65.3	30.6	121	97.2	2.8	—	—	—	71
23	—	—	1.0	62.9	36.1	97	99.1	.9	—	—	—	226

Table I presents the frequency of the different classes of intersexes in the cultures of the female-type and the male-type lines of triploids in each generation of the selection experiment. The frequency of the different classes of intersexes is given in percentages of the total number of intersexes classified in a given generation. The total numbers of intersexes studied in each generation are presented in the columns marked n. As seen from Table I, the selection did affect the type of intersexes produced by the triploid females. The selection was started with a stock of triploids producing all the five classes of intersexes, classes 1 and 2 (male type) being considerably more frequent than classes 4 and 5 (female type). But as early as in the ninth generation, the line producing male-type intersexes gave practically only male-type intersexes (classes 1 and 2), and in the twentieth generation only extreme male-type intersexes (class 1). The selection toward femaleness was not so effective, probably because the initial stock had more male than female tendencies. However, in the twenty-third generation the cultures of the female-type line gave practically only female-type intersexes (classes 4 and 5). The cultures of both lines were kept, of course, under similar conditions, mostly in an incubator at 26° to 27°.

The existence of genetical factors modifying the type of the intersexes is, therefore, evident. It is, however, desirable to prove that these factors are associated with particular chromosomes, *i.e.*, behave as regular genes. The experiment having this as a goal was planned as follows: Males heterozygous for the second-chromosome dominant gene Curly (Cy, wings curved upwards) and heterozygous for the third-chromosome dominant gene Stubble (Sb, short bristles) were crossed to wild-type females from the male-type line of triploids. In the F, generation Cy Sb males were selected and again crossed to wild-type females from the same line. In F_2 generation Cy Sb females were obtained, which, as seen from their pedigree, had both their X-chromosomes coming

from the male-type line. Such females were crossed to males from the line of triploids producing female-type intersexes (female-type line). In the next generation Cy Sb males were selected. The genetical constitution of these males is as follows. They have the X-chromosomes coming from the male-type line and Y-chromosomes coming from the female-type line. Furthermore, they have a second chromosome carrying Cy and the third chromosome carrying Sb coming from the original Cy Sb male, and another second and another third chromosome coming from the female-type line of triploids.

Such Cy Sb males were crossed to triploid females from the male-type line, and the intersexes found in the offspring of this cross were classified according to the presence or absence of the genes Cy and Sb, as well as according to the five classes of intersexes (see above). The results are presented in Table II.

TABLE II

TYPE OF THE INTERSEXES IN THE PROGENY OF TRIPLOID FEMALES FROM THE MALE-TYPE LINE CY SB MALES OF THE GENETICAL CONSTITUTION DESCRIBED IN TEXT

	1	2	3	n
Wild type	78.4	21.6	—	111
Cy	79.6	19.4	1.0	98
Sb	95.7	4.3	—	115
Cy Sb	95.9	4.1	—	98

No intersexes manifesting both the characters Cy and Sb have received either the second or the third chromosomes coming from the female-type line. As seen from Table II, nearly 96 per cent. of them belong to the extreme male type (class 1). No shift in the direction of femaleness is observed in the Cy Sb intersexes as compared with control cultures of the male-type line (see 23-d generation, Table I). It may be concluded that the Cy-carrying second chromosome and the Sb-carrying third chromosome do not produce a shift in the type of

the intersexes in the direction of femaleness. The intersexes manifesting only Sb have received a second chromosome coming from the female-type line. In spite of this, Sb intersexes are of the same type as the Cy Sb intersexes (Table II). Hence, the second chromosome coming from the female-type line is not the carrier of the genes responsible for the female tendency observed in this line.

The Cy-intersexes (Table II) received a third chromosome coming from the female-type line. Less than 80 per cent. of them belong to the extreme male-type (class 1); the remaining 20 per cent. show some reduction of the male genitalia (class 2). In other words, the third chromosome coming from the female-type line produces a shift in the average type of the intersexes toward femaleness. This conclusion is supported by the result of the investigation of the wild-type intersexes (Table II) which are, on the average, of the same type as the Cy intersexes. The wild-type intersexes have received both the second and the third chromosome coming from the female-type line. If the genes modifying the type of the intersexes were distributed proportionally in the second as well as in the third chromosome, the wild-type intersexes would be expected to average considerably more female-like than the Cy intersexes. But since the wild-type and the Cy intersexes are similar in respect to the average sexual type, the genes modifying the type of the intersexes are localized chiefly in the third chromosome.

It must be emphasized, in order to avoid misunderstanding, that the result just described can not be interpreted as meaning that the third chromosome of *Drosophila melanogaster* is the exclusive bearer of the genes modifying the sexual characters. In our experiment we were able to detect the localization only of those modifiers which are strong enough to produce a noticeable effect when present in one dose against two doses of their allelomorphs (all the intersexes shown in Table II have

received two second and two third chromosomes coming from their mothers, *i.e.*, from the male-type line, and only certain ones of them have received one second and one third chromosome coming from the female-type line). Furthermore, any genes localized in the X-chromosome and modifying the type of the intersexes could not be detected in our experiment. The difference between the male-type and the female-type lines of triploids is probably due to a cumulative effect of many modifying factors localized in the different chromosomes, and only some of the most effective of these factors have been found to lie in the third chromosome.

Theoretically, the development of an individual into a female, an intersex or a male depends upon the variation of the balance between the female-determining genes (localized chiefly in the X-chromosome) and the male-determining genes (located chiefly in the autosomes). In other words, femaleness or maleness depends upon variations of the same factors. The difference between the sexes is quantitative in nature. If so, the factors modifying the type of the intersexes toward femaleness or toward maleness may be expected to produce some similar effect on the normal sexual forms. For instance, the males from the line of triploids producing female-type intersexes might be expected to possess some slight indications of the beginning of intersexuality. Likewise, the females from the line producing male-type intersexes might be expected to be slightly intersexual. However, a careful inspection of the males and females from the male-type and the female-type lines failed to show the existence of visible differences between the individuals from the different lines.

The failure of the factors modifying the type of the intersexes to affect the sexual characters of the normal males and females must be interpreted as due to the fact that in the intersexes the critical threshold for the modification of the sexual characters is comparatively low, while in the normal males and females the threshold is

comparatively high. The intersexes in *Drosophila melanogaster* have the genic balance intermediate between the genic balance found in the normal sexual forms. However, a slight shift of the genic balance in either direction produces a striking effect on the sexual characters of the intersexes, but it is not strong enough to surpass the necessary threshold in females and in males.

In other animals, for instance, in Amphibia (Witschi, 1929, and earlier papers) or in *Lymantria dispar* (Goldschmidt, *l. c.*), the threshold for the modification of the sexual characters is far lower, or the modifiers of the genic balance are far stronger, than those discovered so far in *Drosophila melanogaster.* Indeed, in *Drosophila simulans,* a species closely related to *melanogaster,* an autosomal recessive gene is described by Sturtevant (Sturtevant, 1921) which transforms females into intersexes. Although the intersexual females discovered in *Drosophila simulans* are very different morphologically from any known type of triploid intersexes in *Drosophila melanogaster,* the gene producing them may be considered as an example of strong modifiers of the genic balance.

The type of the triploid intersexes in *Drosophila melanogaster* may be modified, however, also by some environmental factors. It was noticed during the course of the selection experiments described above that the cultures developing outside of the incubator gave an average type of intersexes different from that in the cultures developing in the incubator. An investigation of the influence of temperature on the type of the intersexes was, therefore, undertaken.

Several batches of the triploid females and diploid males from the female-type line, twenty females in each batch, were transferred once in every twenty-four hours into a new culture-bottle provided with a standard amount of food. The bottles with the eggs laid in them during the twenty-four-hour period were transferred to one of the four incubators immediately after removal of the parents. Bottles with eggs laid by the triploid fe-

males from the male-type line were secured by a similar method. The temperature in the incubators was kept at 15°, 20°, 24° and 28°, respectively. The regulation of temperature in all the incubators, except one, was exact enough during the whole course of experiment (± ¼°). In the 15° incubator the temperature twice fell to 11° for several hours. The intersexes found in the cultures developed at each of the four temperatures were examined

TABLE III

INFLUENCE OF TEMPERATURE ON THE TYPE OF THE INTERSEXES

t°	Female-type line						Male-type line					
	1	2	3	4	5	n	1	2	3	4	5	n
15°	—	56.8	37.5	5.7	—	88	96.6	3.4	—	—	—	29
20°	—	14.0	56.9	26.8	2.3	86	100.0	—	—	—	—	149
24°	—	2.2	18.0	62.9	16.9	89	98.1	1.9	—	—	—	208
28°	—	—	—	26.2	73.8	61	90.1	9.5	.4	—	—	283

separately and recorded as to type. The results are presented in Table III (the frequency of the different classes of intersexes is given in percentage of the total number of intersexes examined in the cultures of a given series; these total numbers are presented in the column marked n).

More than one half of the intersexes which developed at 15° in the cultures of the female-type line belonged to class 2, *i.e.*, to the male-type, though none of them had well-developed male genitalia. On the other hand, no intersexes of the extreme female-type were produced at 15°. At 20° most of the intersexes belonged to class 3 (intermediate type). At 24° the intersexes belonging to class 4 were predominant, and a considerable number of the extreme female-type intersexes was produced. Finally, at 28° most of the intersexes belonged to the extreme female type (class 5), and no intersexes of the male or of the intermediate types (classes 1 to 3) were produced. In the cultures of the male-type line the effect of temperature is not so striking as in the female-type line. How-

ever, a shift in the direction of femaleness is obvious if the cultures of the male-type intersexes producing line which developed at 28° are compared with those developed at lower temperatures.

It may be concluded that the rise of the temperature at which the development of the intersexes took place resulted in a considerable shift in the average type of the intersexes toward femaleness. In other words, the moment of the reversal of the development occurs relatively late at lower temperatures, and relatively early at high temperatures.

LITERATURE CITED

Bridges, C. B.
1921. "Triploid Intersexes in *Drosophila melanogaster*," Science, 54: 252–254.
1922. "The Origin of Variation in Sexual and Sex-limited Characters," AMER. NAT., 56: 51–63.
1923. "Aberrations in Chromosomal Materials," *Eugenics, Genetics and the Family*, 1: 76–81.
Dobzhansky, Th., and C. B. Bridges.
1928. "The Reproductive System of Triploid Intersexes in *Drosophila melanogaster*," AMER. NAT., 62: 425–434.
Goldschmidt, R.
1920. "Untersuchungen über Intersexualität," *Zeit. indukt. Abst. und Vererbungsl.*, 23: 1–199.
1927. "Physiologische Theorie der Vererbung," Berlin.
Morgan, T. H., C. B. Bridges and A. H. Sturtevant.
1925. "The Genetics of Drosophila," *Bibliographia Genetica*, 2: 1–262.
Sturtevant, A. H.
1921. "Genetic Studies on *Drosophila simulans*. III. Autosomal Genes. General Discussion," *Genetics*, 6: 179–207.
Witschi, E.
1929. "Bestimmung und Vererbung des Geschlechts bei Tieren," "Handbuch der Vererbungswissenschaft," 2, L. 10, Berlin.

HIBERNATION OF THE THIRTEEN-LINED GROUND SQUIRREL, *CITELLUS TRIDECEMLINEATUS* (MITCHILL)

IV. Influence of Thyroxin, Pituitrin and Desiccated Thymus and Thyroid on Hibernation[1]

DR. GEORGE EDWIN JOHNSON AND
VIRGINIA BRANDS HANAWALT

Kansas Agricultural Experiment Station

Introduction

The possibility of any relationship between endocrine organs and hibernation has been the cause of some investigation and also speculation. Gemelli (1906) made histological examinations of the pituitary gland of the adult marmot before, during and after hibernation. He reported that the posterior lobe was unchanged but that there was a great decrease in number of "cianofile" cells during hibernation. Crowe, Cushing and Homans (1910) noted similarities between hibernation and a condition of lowered metabolism following hypophysectomy in dogs. Cushing and Goetsch (1915) found adiposity and a lowering of temperature, respiration and pulse associated with disease of the pituitary in humans. In two patients who had tumors of the hypophysis the injections of extracts made from the whole hypophysis temporarily improved the condition of drowsiness and somewhat subnormal temperature. They also examined histologically the ductless glands of three torpid and four active woodchucks and found evidence of decrease in size of the glands in the dormant state, especially in the anterior lobe of the pituitary, where there was a shrinking of both nuclear and protoplasmic substance. On the basis of

[1] Contribution No. 116 from the department of zoology, Kansas State Agricultural Experiment Station, Manhattan, Kansas. Earlier papers in this series deal with conditions in hibernation (Johnson, 1928) and waking from hibernation (Johnson, 1929a, 1929b).

their observations Cushing and Goetsch concluded that hibernation may be ascribed to a seasonal physiological wave of pluriglandular inactivity.

Mann (1916) made a histological study of the ductless glands of active and torpid *Citellus tridecemlineatus tridecemlineatus.* While some changes were found, he did not consider these related to the seasons constantly enough for definite conclusions. The removal of gonads, thyroids or adrenals did not prevent hibernation. It would appear to the present writers, however, that removal of glands would be more likely to hasten, rather than prevent, entrance into hibernation.

Working with hedgehogs Adler (1920a) found that subcutaneous injections of extracts of thyroid, thymus and suprarenals would usually produce waking from hibernation, and an extract of the anterior lobe of the pituitary increased respiration considerably. Physiological salt solution and extracts from the pancreas, epiphysis and mammary glands did not produce waking. He also found histological indications of lack of secretion in the thyroid in hibernating bats and hedgehogs (flattened epithelial cells and a tannic acid fast staining reaction with specific colloid dyes) and of excessive secretion of colloid just after waking in the spring (tall epithelial cells and fuchsinophil staining reaction of the colloid with specific colloid dyes). Adler concluded that hibernation is the result of hypofunction of the thyroid gland and probably also of the adrenals and the hypophysis. Adler (1920b) reaffirmed these conclusions and reported the production of partial waking with thyroid extract and protein producing amines in torpid hedgehogs whose heat-regulating center had been removed and whose sympathetic nervous system had been deadened with ergotoxin. From this he concluded that the thyroid extract and other substances which produced waking did so chiefly by increasing the oxidative process at the periphery of the animal and not wholly by action on the heat-

regulating center or on the sympathetic nervous system. More recently this work has been summarized by Adler (1926).

Rasmussen (1921) in histological work on thirty-two adult woodchucks (marmots) found no change in weight or histological structure of the hypophysis in hibernation as compared to the condition in the fall before hibernation. He found indications of increased activity of the gland in the spring.

Schenk (1922) confirmed Adler's work, finding the same extracts to produce a rise in metabolism and sometimes waking in torpid hedgehogs. Zondek (1924) produced waking not only with the extracts which Adler used but with others also, and even with a physiological salt solution of a temperature 8° C. or more above the rectal temperature of the animal, and concluded that Adler produced waking not by a specific substance injected but by the warmth of the material injected.

In a speculative article based upon the work of Adler and of Zondek, Koelsch (1925) considered that internal conditions and not external conditions, such as cold and scarcity of food, determine hibernation. He believed that hibernation could not be produced by cold in the summer. That this is a mere assumption has been conclusively proved by records of hibernation in the summer in this laboratory.

With an air of certainty, which might well be envied by the laboratory worker, Berman (1922), in a semi-popular book dealing with the relation of glands to personality, attributes hibernation to a seasonal wave of inactivity of the pituitary gland. Reports like those of Berman and Koelsch and the lack of definite proof that hibernation is related to a condition of the endocrine organs led to the present work, which was begun in 1925–26 by the junior author under the supervision of the senior author and completed by the latter in 1926–27.

Since this work was done, a rather extensive histological study of the endocrine organs in relation to hibernation by Coninx-Girardet (1927) has appeared. She reported a reduction in number of cells in the thymus in winter, a gradual decrease in size of the hibernating gland and a more open grouping of the cells of the hypophysis in winter, the basofils increasing in spring up to the time of rut and decreasing in winter. In the thyroid her findings resemble those of Adler (1920a, 1926) for the follicular epithelial cells were cubical in the spring but were flattened in summer and fall. The colloid was fuchsinophil (indicating active functioning) in the spring but tannic acid fast (indicating inactivity of the thyroid) in the winter. The blood-vessels enlarged and took on the winter condition by the middle of November.

Materials and Methods

In attacking the problem of the possible influence of the ductless glands on hibernation it appeared that if hibernation resulted from the inactivity of one or more glands that the presence in the body of excessive amounts of the secretion of the gland or glands concerned should tend to prevent hibernation.

Feeding of desiccated thymus, thyroid, anterior pituitary and posterior pituitary gland substances procured from Parke, Davis and Company was tried at first. A definite quantity of gland product was made into a paste (with corn syrup, glycerine, water, saccharin, soda and starch) which was made to adhere to a certain number of grains of wheat by stirring followed by drying. In a single experiment three ground squirrels were used for each kind of gland product. Three others were used as controls, which were under the same conditions except they were fed no gland products. In each gland group of three animals, two were fed relatively small doses and one was fed a large dose. These doses ranged from 20 to 120 times[2] the dose a human would receive per

[2] Thymus dose $\frac{1}{2}$ to $\frac{4}{5}$ gr., or 20 to 33 times comparative human dose; thyroid dose $\frac{1}{4}$ to $\frac{2}{5}$ gr., or 31 to 52 times comparative human dose;

pound of body weight by following the directions furnished by the manufacturers. Most of the animals ate most of the grains of wheat fed them for a week before they were removed to a cold room. They were left in the cold room of about 4° C. for seven days and fed and observed daily. Failure of a few of the animals to feed well in the cold room should not vitiate the results since the comparative dosage was so high. The experiments were repeated until the numbers of animals shown in Table I had been used. All but six of the animals used were *Citellus tridecemlineatus pallidus* Allen.

As the feeding experiments may be considered preliminary to the injection work and as they produced no effect it was considered that some of the gland substances may have been destroyed by the digestive system instead of being absorbed. For this reason it was decided to continue the work by injection of thyroxin and pituitrin, extracts of the thyroid and posterior pituitary respectively, since both these extracts could be purchased in potent form.

Pituitrin was procured in sterile condition in 10 cc vials from Parke, Davis and Company. Thyroxin was secured in crystalline form in 10 milligram vials from E. R. Squibb and Sons, and a solution made according to their directions, using 10 cc sterile physiological salt solution and one drop of sodium hydroxide. As a further precaution against infectious organisms the container was then placed in boiling water for several minutes.

Preliminary experiments were carried out to discover what effect the extracts might have on such a small animal as the ground squirrel. Very small doses were administered at first, but as no disastrous effects resulted, greater quantities were administered until in the case of thyroxin the dose adopted was 1 mgm, or 225 times the comparative human dose (taking 2 mgm as a

anterior pituitary 1⅛ to 2⅝ gr., or 40 to 81 times comparative human dose; posterior pituitary 1/25 to 4/25 gr., or 30 to 120 times comparative human dose.

dose for a 150-pound person and the weight of the ground squirrel as 1/3 pound) and in the case of the pituitrin the dose adopted was 1 cc or 450 times the relative human dose (taking 1 cc as the dose for a 150-pound person and the weight of the animal as 1/3 pound). These doses permitted using up all the extract prepared, or in a vial, at one time, thereby eliminating danger of its spoiling before another time of injection. For each animal injected with an extract, referred to as an experimental animal, there was a control animal practically always of the same sex and weight which was injected with an equal amount of physiological salt solution. The control was always kept in an adjacent cage under the same conditions as the experimental animal. Injections were made intraperitoneally. The hypodermic syringe and needle and the ventral body wall were washed with 70 per cent. alcohol to destroy pathogenic organisms. Infection occurred in only one case, an animal injected with 2 cc pituitrin.

After injection with pituitrin the animals were placed in portable 10 by 6 by 6 inch wire cages in a cold room or usually in an automatic refrigerator with a fairly constant temperature of about 8° and never above 13° C., except for short rises when the door was opened for daily observation and feeding. In case of injection with thyroxin the animals were kept in a room of about 16° C. for five days before being placed in the refrigerator, because thyroxin does not begin to act for three or four days and persists for fifteen days, the maximum effect being about the tenth day, according to E. R. Squibb and Sons.

The temperature of the refrigerator and the conditions of the squirrels were recorded daily, whether torpid, partly torpid, stupid or awake. There is no sharp line of distinction between these terms, but in general the torpid animal is distinctly cold to the touch and breathes very slowly (perhaps two respirations per minute); the partly torpid animal is also cold but has a higher rate of respi-

ration and is disturbed during observation, raising the head and perhaps opening the eyes; the term stupid applies to an animal which is rather inactive though breathing almost normally.

Each animal was fed a small handful of oats and some green alfalfa daily.

RESULTS

No marked effect on entrance into hibernation was noted in the feeding experiments. An examination of Table I shows that the thymus and thyroid fed animals

TABLE I

FEEDING DESICCATED GLAND SUBSTANCES TO DETERMINE THEIR EFFECT ON HIBERNATION

	Experiments from October to December, 1925			Experiments from January to March, 1926		
	Number of animals	Per cent. days before hibernation	Per cent. days in hibernation	Number of animals	Per cent. days before hibernation	Per cent. days in hibernation
Thymus	12	32	53	15	26	49
Thyroid	9	32	58	14	28	51
Anterior pituitary	12	41	51			
Posterior pituitary	10	40	52			
Controls	12	41	50	14	30	49

Six animals were *C. t. tridecemlineatus;* the others, *C. t. pallidus.*

in 1925 went into hibernation about 9 per cent. (of the days in the refrigerator) earlier than the two pituitary groups and the control group, suggesting that the thymus and thyroid glands aid in the production of hibernation. However, in the experiments in 1926 there was no difference between these gland groups and the control animals, so that the differences in the earlier experiments were probably due to chance variation in tendency to hibernate.

Twelve pituitrin injection and eight thyroxin injection experiments were performed, each experiment usually including ten experimental animals and ten controls. Since the experiments were intended to measure the influence of the extract upon the entrance into hibernation, the time in days before going into hibernation was divided by the total number of days the animal was in the refrigerator to get the per cent. of days before hibernation. In other experiments[3] it was found that this method gave results which had almost the same meaning as the per cent. days in hibernation did. Table II gives the

TABLE II

EXAMPLES FROM INTRAPERITONEAL INJECTION EXPERIMENTS

Thyroxin experiment number 3 June 12–26, 1926				Pituitrin experiment number 6 July 3–10, 1926			
Thyroxin		Saline (Control)		Pituitrin		Saline (Control)	
Animal number *Ctp**	Per cent. days before hibern.	Animal number *Ctp*	Per cent. days before hibern.	Animal number *Ctp*	Per cent. days before hibern.	Animal number *Ctp*	Per cent. days before hibern.
341	20	340	100	102	100	252	14
343	20	342	50	269	100	249	29
345	50	344	20	429	57	247	100
347	10	346	70	430	100	431	100
349	70	348	100	432	57	433	57
351	50	350	10	292	57	318	14
353	100	352	30	320	100	300	71
355	40	354	70	434	43	435	29
357	100	356	70	319	43	438	14
359	80	358	50	436	28	437	71
Average	54		57		69		50

Explanation.—All the animals in one experiment were in the refrigerator the same length of time (7, 10 or 14 days). The number of days which passed before an animal became torpid was divided by the total number of days it was in the refrigerator in that experiment, thus Ctp341 went into hibernation after two days in the refrigerator, Ctp340 failed to hibernate during the ten days in the refrigerator, etc.

* *Citellus tridecemlineatus pallidus* Allen.

[3] Abstracts given in *Anat. Rec.*, 31: 337; 37: 125.

results of one thyroxin and of one pituitrin injection experiment. These are given to show something of the variation as to days before going into hibernation. In these two experiments some animals did not go into hibernation, whereas in some experiments a few went into hibernation on the day the animals were placed in the refrigerator. This variation among animals and also the variation among results of different experiments made it very evident that a number of experiments were necessary. The lack of definite correlation between injection of either pituitrin or thyroxin and tendency to hibernate is quite evident in Table III. Statistical treatment of the

TABLE III

SUMMARY OF INJECTION EXPERIMENTS GIVING AVERAGES OF PER CENT. OF DAYS BEFORE HIBERNATION

Thyroxin experiments					Pituitrin experiments				
	Thyroxin		Saline (control)			Pituitrin		Saline (control)	
Date 1926	No. of animals	Ave. per cent.	No. of animals	Ave. per cent.	Date 1926	No. of animals	Ave. per cent.	No. of animals	Ave. per cent.
May 6–20	3	67	5	55	Apr. 28–May 6	8	35	8	25
May 14–21	10	45	8	50	May 20–30*	9	60	10	62
June 12–16	10	54	10	57	May 24–June 7*	10	63	11	48
June 21–July 6	9	48	9	39	June 14–21*	9	47	10	58
June 21–July 6	7	39	7	71	June 14–21*	10	58	11	52
July 9–24	8	54	8	40	July 3–10	10	69	10	50
Nov. 2–19	9	18	9	24	July 15–22	11	56	11	65
Dec. 8–22	10	43	10	31	July 31–Aug. 14	10	46	10	49
					Sept. 3–18	9	40	10	31
					Oct. 7–20	10	32	9	21
					Oct. 23–29	10	17	10	20
					Nov. 22–Dec. 6	10	2	10	59
Totals	**66**		**66**			**116**		**120**	
Averages		44		45			44		46

* Animals placed in two-quart cans with lids in which were punched four 8 penny holes, since this procedure had previously been found to produce hibernation more rapidly than leaving the animals in open cages (Johnson, 1925).

figures shows that the actual difference divided by the probable error was only 0.63 for the pituitrin experiments and would be less for the thyroxin injection experiments, results which show that these extracts have no significant influence on hibernation, since to be significant the number would have to be 4 or more instead of 0.63.

As another measure of the power of these extracts to prevent hibernation a tabulation was made of the number of animals torpid, partly torpid, stupid and normal one day after injection and placing in the refrigerator in the pituitrin experiments, or one day after placing in the refrigerator in the thyroxin experiments where injections were given five days before placing in the refrigerator. No difference between the injected animals and the saline controls was found in either the pituitrin or thyroxin experiments. It would be at this time that the inhibitory quality of the extracts should be most effective, for the natural tendency to hibernate is not great during the first twenty-four hours in the cold, and a slight stimulation on the part of the extract might be expected to keep the animal awake.

It should be stated that there was a marked loss of weight in some of the animals during the period in the refrigerator at first because the animals were not fed as much as when not in the refrigerator. However, since control and experimental animals were under the same conditions this loss of weight should not affect the one group more than the other.

Discussion

While the anterior and posterior pituitary feeding experiments probably are not significant because these substances may be digested before they are absorbed, thyroid and thymus feeding are common experimental procedures and thyroid feeding at least is known to be effective in humans. For this reason the thyroid feeding experiments should be considered valid and tending to show that the thyroid has no influence on hibernation. If

thymus feeding results in any endocrine effect in other animals then it would seem that feeding it to twenty-seven animals should have a significant effect if it is a predominating factor in the production or the prevention of hibernation.

If unusual activity of the thyroid or of the posterior pituitary tends to prevent hibernation then it appears there should have been a decided effect shown in our injection experiments because the dosages used were very large and because the number of animals used was large (66 in the thyroxin injections, besides the 23 in the thyroid feeding; and 116 in the pituitrin injection work).

As a result of this work and because other injection work (Adler, 1920a; Schenk, 1922) has been placed in doubt by Zondek (1924) it is evident that physiological work to date does not support the idea that hibernation is produced by inactivity of the thyroid or of the posterior pituitary.[4] Any theory that thyroid activity is antagonistic to hibernation would at present have to rest on histological work. Adler (1920a) and Coninx-Girardet (1927) found changes in follicular epithelium and in the colloid of the thyroid, indicating greater functional activity, in the spring after hibernation, yet this might be an effect of waking rather than a cause. While the work of Cushing and Goetsch (1915) led logically to the idea that hibernation is produced by pluriglandular (including thyroid) inactivity, it must be noted that their histological investigations involved only seven woodchucks, too small a number for valid conclusions. It must also be noted that Mann (1916) found no consistent changes in any of the ductless glands in relation to the time of hibernation. Even if a seasonal change were demonstrated in these glands this would not necessarily have a causative relation to hibernation. In fact, the only conclusive evidence must be procured from physiological experimentation, and this does not now give evi-

[4] The anterior pituitary will be considered in a later paper.

dence for any influence on hibernation exerted by the thyroid or the posterior pituitary.

Summary

The feeding of desiccated thymus and thyroid in large doses to twenty-seven and twenty-three animals, respectively, resulted in no definite increase or decrease in tendency of ground squirrels to hibernate when subjected to cold temperatures. The injection of thyroxin and pituitrin in doses which were, respectively, 225 and 450 times the human dose on the basis of relative weights, produced no change in tendency to hibernate in 66 and 116 animals, respectively, in comparison with the same number of controls. The animals were subjected to cold temperatures after the injections.

The experiments indicate that the secretions of the thymus, thyroid and posterior pituitary are not important agents in the production or prevention of hibernation.

LITERATURE CITED

Adler, L.

1920a. "Schildrüse und Wärmeregulation" (Untersuchungen an Winterschläfern), *Arch. Exper. Path. u. Pharmak.*, 86: 159–224.

1920b. "Über den Angriffspunkt der Blutdrüsen-hormone bei der Wärmeregulation," *Arch. Exper. Path. u. Pharmak.*, 87: 406–423.

1926. "Der Winterschlaf," *Handbuch der normalen und pathologischen Physiologie*, 17: 105–133.

Berman, L.

1922. "The Glands Regulating Personality." The Macmillan Co., New York.

Coninx-Girardet, Berta

1927. "Beiträge zur Kenntnis innersekretorischer Organe des Murmeltieres (*Arctomys marmota* L.) und ihrer Beziehungen zum Problem des Winterschlafes," *Acta Zool.*, 8: 161–224.

Crowe, S. J., Cushing, H., and Homans, J.

1910. "Experimental Hypophysectomy," *Bull.* the Johns Hopkins Hosp., 21: 127–169.

Cushing, H., and Goetsch, E.

1915. "Hibernation and the Pituitary Body," *Jour. Exp. Med.*, 22: 25–47.

Gemelli, A.

1906. "I processi dell secrezione dell 'ipofisi dei mammiferi," *Arch. Sci. Med.*, 30: 521–550.

Johnson, G. E.

1925. "Some Conditions Affecting Hibernation, etc.," *Anat. Rec.*, 31: 337.

1928. "Hibernation of the Thirteen-lined Ground Squirrel, *Citellus tridecemlineatus* (Mitchill); I. A Comparison of the Normal and Hibernating States," *Jour. Exp. Zool.*, 50: 15–30.

1929a. "Hibernation, etc.; II. The General Process of Waking from Hibernation," AM. NAT., 63: 171–180.

1929b. "Hibernation, etc.; III. The Rise in Respiration, Heart-beat and Temperature in Waking from Hibernation," *Biol. Bul.*, 57: 107–129.

Koelsch, A.

1925. "Der Mechanismus des Winterschlafs." *Kosmos*, (Heft 1), 14–17.

Mann, F. C.

1916. "The Ductless Glands and Hibernation," *Am. Jour. Physiol.*, 41: 173–188.

Rasmussen, A. T.

1921. "The Hypophysis Cerebri of the Woodchuck (*Marmota monax*) with Special Reference to Hibernation and Inanition," *Endocrin.*, 5: 33–66.

Schenk, P.

1922. "Über den Winterschlaf und seine Beeinflussung durch die Extrakte innersekretorischer Drüsen," *Pflügers Arch. ges. Physiol.*, 197: 66–80.

Zondek, B.

1924. "Untersuchungen über den Winterschlaf," *Klin. Wchnschr.*, 3: 1529–1530.

SHORTER ARTICLES AND DISCUSSION

BIOLOGICAL NOTES ON THE MOSS-MITES

In Sellnick's synopsis of the Oribatoidea in "Die Tierwelt Mitteleuropas," 185 species out of 220 whose habits are recorded occur in moss. It is well known among specialists in this group that the most certain way of securing species is by collecting moist moss. Furthermore, there is no other group of mites which does occur in moss except in very limited numbers. In fact, one may find more species of Oribatoidea in moss than of any other single group of minute animals. Thus not only is moss the typical habitat of the Oribatoidea, but they are typical inhabitants of moss. Therefore, as there is objection to the term beetle-mites for this group, they not being parasitic on beetles, the writer proposes to use the term moss-mites.

Although many papers on ecology briefly discuss fired land as a habitat, usually as a successional factor, I know of no report on animals found on land immediately after, or a year after, a fire. Oribatoidea have been found on suburban land (empty lots within the limits of New York City on Long Island) which has been repeatedly burned for a period of many years. Yet Oribatoidea were fairly common. For instance: on March 2, an hour of picking over burned leaf-humus yielded four specimens representing three species; March 8, dead sticks, similar situation, yielded 10/6; dead leaves, similar situation, 4/2; branch (about an inch in diameter) charred at both ends, moist, punky area between ends yielded 35/3; April 28, decaying and charred sticks among leaves of burned-over tract, 12/2. Most of these species were less than a millimeter in length. To what extent collembola, myriapoda, mollusca, etc., will endure the "swift" passage of a woodland fire, either as eggs or adults, is still to be determined.

An important factor concerning distribution, which has rarely been referred to (I know only of Halbert, in his report on the terrestrial and marine Acarina, Clare Island survey), is the influence of birds as distributors—not as direct carriers but as carriers of eggs, spores and adults of small plants and animals on *nest-material*. To what extent Krakatoa has been thus replanted by algae, fungi, mosses and lichens and restocked with some of the above-mentioned groups of animals by sea-birds, ospreys, etc., carrying even large sticks from neighboring islands, is entirely a matter of conjecture but would easily explain the presence of many species thus far accounted for by wind.

To what extent these animals are carriers, if not inoculators, of fungal diseases may be surmised from two facts: (1) some of the species nightly leave the earth to ascend vegetation. Others, though passing their daylight hours on the trees they ascend, are washed off in numbers by pelting downpours and transported some distance before being washed onto the drift line, to ascend new vegetation on return of darkness. (2) In some species, a high percentage of individuals have fungus spores attached to them. This is particularly true of the very rough, tree-dwelling Udetaliodes and of the large-winged Galumninae, which, though smooth, continually lodge spores in their leg-cupboards, and the Phthiracaridae which repeatedly draw spores into their bodies on housing their legs and mouth parts. At least one species has been found whose crop or stomach was crammed with spores, while others have been seen with small numbers of spores thus ingested. *Pediculoidus dianthophilus* (a mite of another suborder) is known to disseminate *Sporotrichum poae* on carnations. When one considers how little is known concerning the distribution of these spores (except by wind) and the important part some beetles have been found to contribute, one may legitimately suspect these animals as a source of infestation if not inoculation.

The effect on the surrounding fauna of dynamite used in blasting on agricultural land seems also to have been overlooked. An observation made by the writer may indicate that it is considerable, especially to terricolous species.

The habit of rapidly vibrating the anterior leg in a vertical direction by a species of Eremobelba observed in the Diamond Mountains of Korea would indicate that the sensory habit *de tatoner* may originate before a structure has been particularly modified for this purpose. The Oribatoidea are eyeless. In other Acarina (with eyes) the anterior pair of legs is modified as a feeler. If the long bristles and other specialized hairs on the legs, especially of the anterior pair, are regarded as particularly tactile, then only one species (probably genus) thus far observed has developed the habit *de marcher en tatonant.*

Two types of mutation have been noticed. Jacot (1929) records two cases of presence of an extra bristle. Throughout the group, where bristles appear they are extremely constant in their occurrence and spring from definite tracts or rows and areas. They may at these predetermined places be present or absent, but rarely do bristles appear elsewhere, and in such cases it is purely an abnormality. For instance, in one case a bristle is

present on one "shoulder" and not on the other, making the animal asymmetrical. In the other case the supernumerary bristle appears on the median line, and from its position and that of the nearest normal bristle it appears to be due to a standard bristle having become two in early embryonic development. Another similar example is figured by Jacot (1929b) where on *one* anal cover an extra bristle shares the area with the normal two bristles, displacing them equidistantly. These thus have all the appearance of "bottle" mutations (but occurred in God's own bottle) or of polydactylism and other such indeterminate, discontinuous meristic aberrations. That they can never become normal or standard seems clear from the fact that they are on one side only or outside the standard tracts or rows, *i.e.*, they are not provided for in the mechanics of the embryological pattern and completed structure. Being out of plan they occur at rare intervals only. The geneticists have been spending a generous degree of time experimenting with this type of waste by-product of the somatoplasm without yet having learned why they are thrown off by life forms or why they are spurned in God's own bottle (and by the systematist).

It should be added that both these cases occurred in what the writer would call an *ornate genus, i.e.*, a group of species which have specialized in (1) ornate sculpturing, (2) the production of such projecting ornaments as warts, spines, horns, etc., and (3) in invaginations and foldings of the body wall or parts. Such genera occur in all groups, even among the Amoebae (Schaeffer, 1926), and are usually considered to be more advanced (specialized) species, possibly of greater age and approaching senescence. By contrast, among the *simple genera, i.e.*, species lacking ornation, even though represented by far more species and individuals, not a specimen was found bearing a "bottle" mutation.

The second type of mutation is where, through a type of syzygy, the lamellae (on the cephaloprothorax) have their ends approximated on the median plane, due to the absence of the intervening area, instead of standing far apart. This has been observed in a specimen of *Parakalumma robustum* (Banks) from Ithaca, New York, and a specimen of *Edwardzetes edwardsii* (Nicolet) from Lausanne, Switzerland. The ecological result is that the two long, tactile, anteriorly directed lamellar bristles have been shifted from a lateral position, where there are already several long tactile bristles, to a median one where there are none. As this modification has been approximated by at least two or

three genera not related to the above, it can hardly be regarded as a case of reversion. As it is a wild mutation and as it has been adopted in some genera, may it not indicate that mutations or somatic changes at times follow definite lines imposed by the mechanics of stresses and strains, by concentration or centralization or other structural modifications, but that they can not become stabilized as specific or generic characteristics until frequent enough to insure a neighboring male and female bearing them the opportunity of beginning a new race?

Thus one may classify mutations as *aberrant* when of such a nature as to lead nowhere and as *directive* if of such kind as to be in harmony with the structure pattern of the group and of no distinct disadvantage.

In this group one finds a series (the Pterogasterines) showing all steps in the development of a lateral ridge into large, thin, movable, veined wings. Although these wings, in the most highly developed group (the Galumninae), act as cupboard doors for enclosing the legs, they may well be used secondarily for gliding from the top of herbs to the ground. Such a descent has not been recorded, which is not strange as it would occur at night or daybreak and resemble the descent of a pollen grain. Were these wings adequately and properly muscled (their bases are quite thick and harbor certain muscle attachments) they might be used for flight. The veining varies in different species and is probably the result of muscle attachments or stay ridges. A study of these structures may help in understanding the development of wings in the Pterygote insects from dorsal lobes as outlined by Snodgrass (1927). Here one finds the development of a prominent structure from a ridge through leg guards and covers nearly to a flight organ—the structure before the function.

ARTHUR PAUL JACOT

SHANTUNG CHRISTIAN UNIVERSITY

LITERATURE CITED

Jacot, Arthur

1929a. "Phthiracarinae of the Northeastern States," *Bul.* Mus. Comp. Zool. (in press).

1929b. "American Oribatid Mites of the Subfamily Galumninae," *Bul.* Mus. Comp. Zool., 69: pl. 2, fig. 7.

Schaeffer, A. A.

1926. "Taxonomy of the Amebas," Car. Inst. Pub. 345, pls. 11, 12.

Snodgrass

1927. "Morphology and Mechanics of the Insect Thorax," Smithsonian Misc. Coll. 80/1, pp. 42–45.